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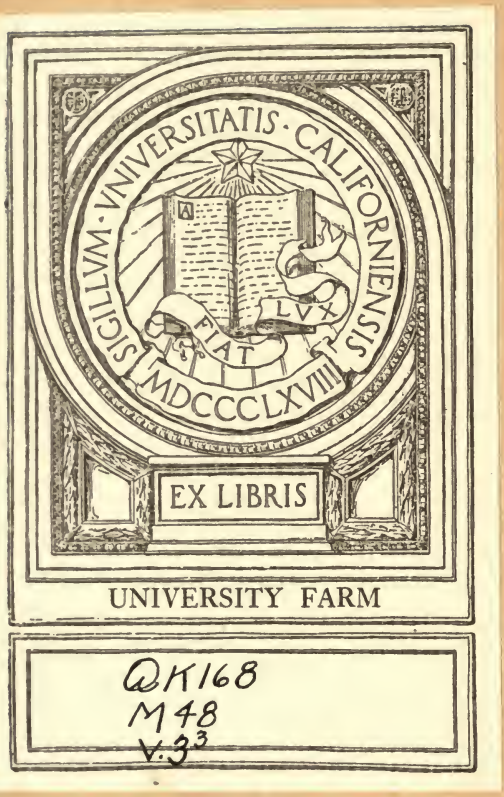


XXIII. THE EMBRYOGENY OF GINKGO.

Harold L. Lyon.

INTRODUCTION.

The account published by Coulter and Chamberlain in 1901 is the most recent work on the embryogeny of *Ginkgo* and may be taken as a summation of our knowledge on the subject up to the present time. They wrote as follows, "Although the embryo of *Ginkgo* is exceptional among Gymnosperms, and of great interest, the details of its development are not sufficiently known. We have been able to secure almost a complete series showing the general outlines of the development, which merely confirms the facts already published. Germination of the oöspore begins, as is usual, among Gymnosperms, with repeated nuclear divisions. These nuclei, however, instead of organizing a parietal tissue as in the Cycads, or a basa[1] group as in the Conifers, proceed to fill the whole cavity of the enlarging oöspore with free nuclei, which is followed by the organization of a compact tissue. In a certain sense this structure would seem to represent the proembryo of Cycads, but it really represents the whole product of the oöspore, in which proembryo, suspensor, and embryo proper are not differentiated. The complete filling of the spore with tissue, and the lack of early differentiation into the great embryonic regions, would suggest a habit more primitive than in either Cycads or Conifers. At the same time, it may be merely a derived character. In any event, the tissue near the base of the spore, which in the other groups develops both suspensor and embryo, shows far greater vigor than the remaining tissue. In the organization of the embryo the whole mass of tissue is involved, and in the absence of a suspensor the embryo invades the endosperm by direct growth.

The two cotyledons are differentiated early in October, and are quite unequal in length. The larger one is two lobed, while the shorter one is cleft halfway down, thus early indicating the bilobed character of the leaf. The two cotyledons are also united at the apex, but the epidermal layers of the two are dis-

tinct where they are in contact. The plumule is very conspicuous between the elongated cotyledons, three or more leaves appearing just behind the stem apex."

In 1872 Strasburger published the first paper dealing with the embryogeny of *Ginkgo*. He determined the general course of events in the formation of the spherical embryo but insufficient material prevented the working out of details. Hirase ('95) studied fecundation and the origin of the spherical embryo. He found that there were eight simultaneous nuclear divisions in the oösperm preceding free-cell-formation. More recently Ikeno (1901) has made a very careful study of the process of fecundation.

The embryo of the seed has been frequently described, but little however seems to be known of its histology. Endlicher ('47) was the first to mention the occurrence of two or more embryos in one seed and recently Cook (1902, 1903) has called attention to polyembryony in *Ginkgo*. Miss Wigglesworth (1903) notes the presence of stomata on the cotyledons. LeMaout and Descaisne ('76) and Masters ('91) figure seedlings of *Ginkgo*. Van Tieghem ('70, '87) and Van Tieghem and Douliot ('88) record observations on the anatomy of the root system. Worsdell ('97) describes the vascular bundle and transfusion tissue of the cotyledon, and Seward and Gowan (1900) contribute some further observations on the anatomy of the seedling.

The remarkable ciliated spermatozoids of *Ginkgo* have received such critical attention from Japanese botanists[1] that no observations on these need be offered here. With the corroborative evidence from the cycads[2] our knowledge of the occurrence and development of ciliated spermatozoids in these Gymnosperms may be considered well established. In the present paper the subjects of oögenesis, spermatogenesis and fecundation will be avoided, the recorded observations dealing solely with the embryogeny. It is purposed to take up and continue the subject where Strasburger left it some thirty years ago.

Material.

The investigations, which are recorded in the following pages, were begun in 1901 on material received from the Botanical

[1] Hirase ('96, '97, '98), Fujii ('98, '99), Miyake ('98, 1902).

[2] Ikeno ('96, '97), Ikeno and Hirase ('97), Webber ('97[1], '97[2], 1901), Lang (1900).

Gardens of the Imperial University of Tokyo through the kindness of Mr. K. Yendo. Later large quantities of excellent material were obtained from the Missouri Botanical Garden, and it is principally from this material that embryological data have been obtained. The writer wishes to express his sincere thanks to Dr. William Trelease who has done every thing possible to facilitate the work. Some valuable preparations have also been obtained from a small number of seeds purchased from Thorburn & Co., seed dealers of New York. The location of the trees which bore these seeds was not determined. The material sent from Japan by Mr. Yendo, amounted to several quarts of seeds containing nearly mature embryos. The embryos were removed from a large number for examination, those shown in Plate XXXV. being some of the number, while many of the seedlings studied were grown from these seeds. Under the directions of Dr. Trelease, cuttings bearing megasporangia were sent from the Missouri Botanical Garden at intervals of a few days throughout an entire season. After the cut ends of the branches had been sealed over with paraffin, they were packed in moist sphagnum, wrapped first with oiled, then with heavy paper and sent by parcel-post. These excellent precautions brought the material to this laboratory in a perfectly fresh condition. Upon arrival it was immediately placed in the various fixing fluids.

Research.

A. The Embryo.

1. *The Protocorm.* — At the completion of free-division the nuclei are quite evenly distributed through the cytoplasm of the oösperm (*fig.* 1), and when the formation of walls between these nuclei is first completed the resulting cells show no marked dissimilarity in shape, size or contents (*fig.* 2). The cells, in the upper two thirds or more of this spherical protocorm, divide only a few times or not at all. Their protoplasmic contents become thin and watery, and they take no part in the organization of the metacormal bud or blastema. The cells in the lower portion of the protocorm divide repeatedly, the relative activity increasing towards the base so that in this region there is organized a small-celled tissue (*figs.* 3, 4). This basal tissue passes over directly into the small-celled meristem of the blastema. With the advance of the metacorm into the body of the gametophyte, the protocormal tissue is forced back through the neck

of the archegonium until it comes in contact with the firm nucellar tissue (*figs. 4–9*). Many of its cells are often crushed by this backward pressure, but in the mature embryo of the seed the protocormal region is still evident (*figs. 11, 34, 36*).

2. *The Blastema.* — The blastema arises directly from the small-celled, basal tissue of the protocorm, and invades the gametophyte as a broad, blunt cylinder (*figs. 4, 5, 6, 8*). The central region of the gametophyte seems to be the least resistant, and from the latterly disposed protocorm, the blastema directs its growth into this central tissue (*figs. 5, 8*). The path, which the embryo is to follow, is marked out for a considerable distance ahead of it by disorganized cells (*fig. 8*).

At first the metacormal bud is throughout meristematic; but very soon there can be distinguished two growth-foci, one directly behind the other, in the axis of the blastema, and only separated from each other by a very few cells (*fig. 5*). The apical growth-focus is the growing point of the stem, and includes the entire apical region of the young metacorm. The second growth-focus is the growing point of the root. It first becomes noticeable through the apparent diverging from this area of the indefinite cell-rows which extend forwards towards the apex of the blastema (*figs. 5, 6*). It also immediately begins to cut off rectangular cells from its end towards the protocorm, which form the characteristic, parallel cell-rows of the central root cap region (*figs. 5–7*). Both growing points arise through a localization of growth activity out of one general meristematic tissue; and hence, from the first are many-celled meristems.

3. *Cotyledons and Leaves.* — The cotyledon primordia originate through a localization of growth-activity in the marginal region of the broad apical meristem (*fig. 6*). They first appear as crescent-shaped mounds of tissue, which push rapidly ahead of the stem apex (*fig. 7*). Each cotyledon has an apical meristem. Of the many embryos from Japan examined, all were dicotyledonous with the exception of two; but among the hundreds of embryos from the Missouri Botanical Garden, which have been studied, over fifteen per cent. were tricotyledons. The more common number of cotyledons is therefore two; but it is by no means constant as is usually stated to be the case. The plumular leaves, whose origin directly follows that of the cotyledons, arise in the same manner as the latter, but have a much more restricted intraseminal growth (*fig. 10*).

4. *Embryonic Tissue-Systems.* — As the blastema grows out from the protocorm, its superficial cells are irregular, and divide by periclinal as well as anticlinal walls (*fig.* 4). This condition prevails even after the stem apex has become quite distinct (*fig.* 5). Later, with the broadening of the apex, which causes a rapid increase of surface, periclinal divisions become less frequent. They, however, continue to occur for some time in the most central region of the stem apex, and can often be detected here in nearly mature embryos. Superficial cells of the cotyledon and leaf-meristems have been found dividing periclinally. In regions, other than meristems, the dermatogen appears to be morphologically distinct from the subjacent tissue.

The plerome and periblem can only be recognized as regional in the embryo, for they are not sharply marked off from each other. The first procambial tissue is differentiated in connection with the cotyledons, passing from these straight back into the body of the embryo (*fig.* 7). The next procambium-strands arise, in like manner, in connection wtth the first plumular leaves; but since these are closer together than the cotyledons, their procambium-strands are closer together. The shape of an embryo's stele is determined by these two sets of procambial strands. Hence, if there are two cotyledons, the stele will be ellipsoidal (*figs.* 13–15); but if there are three cotyledons, it will be triangularly prismoidal (*fig.* 18). At the root-meristem, an ellipsoidal plerome narrows down into a broad wedge (*figs.* 11, 12); while in the same region a prismoidal plerome narrows down into a blunt pyramid. A single procambium-strand enters each cotyledon. As it passes upward in the cotyledon, this strand, usually but not always, divides once. A few protoxylem-elements are often differentiated in the cotyledon-bundles before intraseminal growth stops.

In both plerome and periblem numerous secretory vessels are formed through the breaking down of cells in longitudinal rows (*figs.* 10–12). Many spherical resin-reservoirs also arise in the cortex of the stem, cotyledons and leaves by the disorganization of masses of cells (*figs.* 10–20). In mature embryos the cells of the cortex are packed with starch.

5. *Polyembryony.* — It often happens that when the oösphere of one archegonium of a gametophyte is fertilized, the oösphere of the other is also fertilized and two protocorms develop. Occasionally gametophytes will be found with three archegonia;

and in one such exceptional case three young embryos were found. Of the two embryos often formed in one seed, the more vigorous soon occupies the central, non-resistant tissue of the gametophyte, while the other aborts. In the many seeds examined, only one case has been found (*fig. 29*) where two embryos from different oösperms have developed to maturity in the same gametophyte. Several cases, however, have been met with, where two metacorms have resulted through the production of two blastemata by one protocorm. Figs. 36 and 37 show two such cases; while fig. 9 shows an instance where two blastemata have arisen on one protocorm, and then one has aborted.

6. *The Mature Embryo of the Seed.* — The single embryo figured and described by Seward and Gowan (1900) was, without question, a freak; for it bears little resemblance to a typical embryo. The general description of *Ginkgo* embryos given by Coulter and Chamberlain, as quoted above, was undoubtedly constructed from that of Seward and Gowan; for they describe the same anomalies as constant characters of the embryo.

A very good idea of the embryo of the seed can be obtained through an examination of the photographs of Plates XXXIV–XXXVI. Figs. 23 and 25–28 are typical dicotyledonous, and figs. 22 and 30–34 typical tricotyledonous embryos. Figs. 13–17 are sections of a dicotyledonous, and figs. 18 and 19 sections of a tricotyledonous embryo. Occasionally freakish, deformed embryos are met with, but are no more numerous than is usual in other plants. The one portrayed in fig. 35 is the only one among the hundreds examined which approximated the one described by Seward and Gowan; and the shorter cotyledon of this one was quite entire. The embryo, portrayed in fig. 24, is one of several found showing a condition intermediate between typical dicotyledonous, and tricotyledonous embryos. Fig. 20 is a section of the same embryo. In this case a third leaf was produced at the base of the plumule on the same level as the cotyledons. It was, however, partially enclosed by the functional cotyledons (*fig. 20*), and in length only equalled the plumule. The stele of this embryo was triangularly prismoidal as in the case of typical tricotyledons. Fig. 21 is a section through the cotyledons of an apparently dicotyledonous embryo, which was morphologically tricotyledonous; two cotyledon-primordia having developed conjointly, producing a single

large member. This embryo possessed the stele of a typical tricotyledon. In several similar cases noted, the primordia had assumed independent growth after a period of conjoint development, so that the cotyledons were not united throughout their entire length (*fig. 35*).

Five leaf-primordia are usually to be distinguished on the plumule of a mature embryo. In a dicotyledonous embryo, the cotyledons are often not so truly diametrically opposite each other, as are the two following leaves; one of the latter being regularly larger than the other, and occupying more space between the cotyledons (*figs. 15–17*). Beyond the cotyledons and first pair of plumular leaves, a decussate arrangement does not obtain in the embryo (*fig. 17*). In a tricotyledonous embryo, the leaves are arranged spirally and have an indeterminate divergence (*fig. 19*).

In a mature seed the embryo extends through one half to two thirds the length of the gametophyte. The cotyledons are of equal length; often slightly reflexed at their tips (*figs. 24–27*), and when the embryo is removed from the seed, they usually separate of themselves. Frequently, a considerable mass of disorganized gametophytic tissue is included between the cotyledons. The stem and root regions and also the proximal halves of the cotyledons are white; the ends of the cotyledons being yellowish or light green in color. The resin-reservoirs are most numerous in the stem and basal portions of the cotyledons, and in fresh embryos stand out as prominent greenish or amber-colored pustules.

When an embryo is placed in a chromic acid fixing solution, the root-cap region turns brown. A section (*figs. 7, 11, 12*) shows this color-change to be limited to the peripheral layer of cells; the change being due to a change in their contents. The fixing-fluid has a similar effect on the contents of the secretory vessels, and also darkens the resin-globules.

B. The Seedling.

1. *Morphogenesis.* — When seeds are placed under favorable conditions for germinating, the hard shell is cracked, at the micropylar end, by the swelling of the gametophyte. Through this opening the body of the embryo is thrust out by the elongation of the cotyledons. The root immediately turns down into the soil (*fig. 39*), and as soon as the stem is free from the

gametophyte it begins to grow in the direction opposite that followed by the root (*fig. 41*). With the elongation of the stem, the plumular leaves are quickly displaced from their original relative positions (*figs. 42–47*). The first two or three leaves directly following the cotyledons do not develop the characteristic blades, but remain small and scale-like (*fig. 47*). The stem soon stops its rapid elongation, and having expanded a rather close crown of leaves at its apex (*figs. 47, 48*), spends the remainder of the first season in strengthening and protecting itself by secondary growth in its tissues.

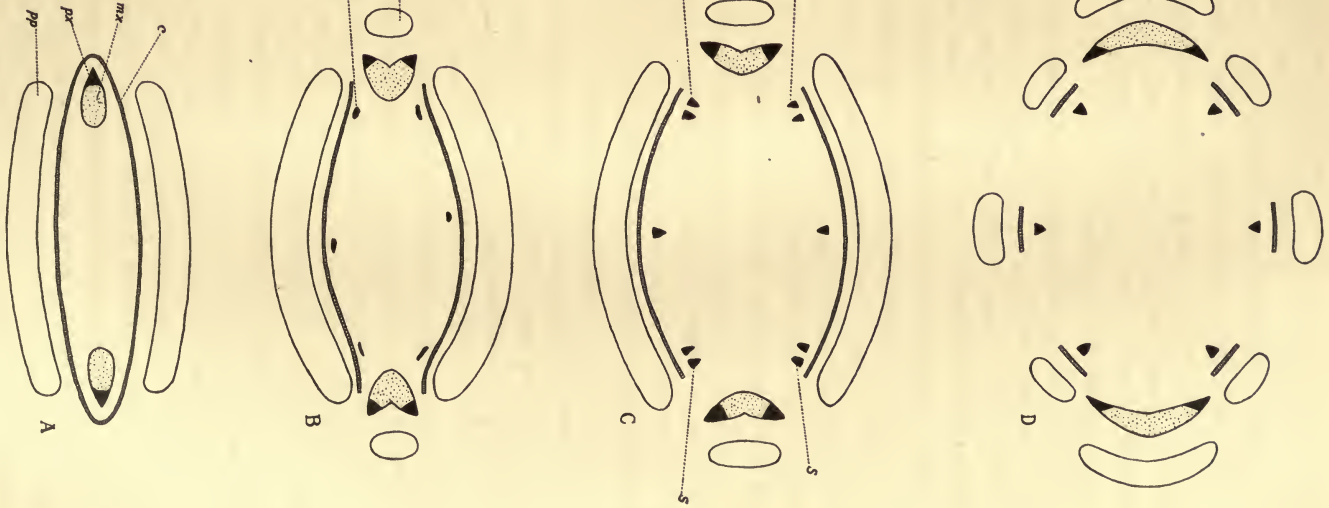
As is the rule among gymnosperms, the primary root of *Ginkgo* persists as the tap-root. During the first season it develops numerous short secondary roots (*figs. 47, 48*).

Upon the germination of the seed, the proximal portions of the cotyledons, which, as we have already noted, are well supplied with resin-reservoirs, protrude from the gametophyte as large, arching petioles (*figs. 41–44*). The portion of the cotyledon, remaining within the gametophyte, enlarges considerable, (*fig. 48*), its greatest diameter being about twice that of a cotyledon of a mature embryo of the seed. The cotyledons persist throughout the first season. Fig. 48 is a photograph of a living seedling at the end of its first summer's growth. It was taken in October, after the seedling had experienced several quite heavy frosts. The remains of the seed were removed exposing the large fleshy cotyledons, which sprang apart of themselves as soon as freed from the tissue of the gametophyte.

A large terminal bud is the only prominent one on the stem during its first winter; and, if uninjured, is the only one to become active during the second season. Fig. 49 is a photograph of a young plant which has made a considerable portion of its second year's growth. The leaves all possess fully expanded blades, and are well distributed along the stem. During its second season the plant develops a much-branched root-system.

2. *Histogenesis.* — As has already been mentioned in discussing the embryo of the seed, the first permanent vascular tissue is differentiated in the cotyledons. As the embryo begins its extraseminal development, differentiation progresses from the cotyledons towards the root-apex. The two bundles of a cotyledon unite eventually to form one of the persistent bundles which extends throughout the length of the root. If the seed-

Diagrams illustrating, in a general way, the stelar structure at various points in the transition-region between root and stem. *A*, below the transition-region; *B*, in the lower transition region; each primary bundle dividing to form the two bundles of a cotyledon trace; C, farther up in the transition region; the four secondary bundles "*s*" later unite with the cotyledon traces; *D*, at the point of exit of the cotyledon-traces; *E*, in the petiole of the cotyledon; the two bundles still closely associated; *pp*, protophloem; *px*, protoxylem; *mx*, metaxylem; *c*, cambium.



ling has two cotyledons, the primary root will be diarch; if it has three cotyledons, the root will be triarch, a condition which Dangeard ('90) has noted in other gymnosperms. In the transition-region between root and stem, considerable diversity of structure prevails among *Ginkgo* seedlings. Yet the numerous, and seemingly very different types can be readily explained as modifications of the one general plan illustrated conventionally in the adjacent diagrams.

Examining serial sections of a seedling of about the age of those shown in figs. 42–46 we find, a short distance above the root-meristem, two xylem-bundles appearing at the ends of the elongated oval stele; and in the sides of the latter, two long arching bundles of protophloem (Diagram A and *fig. 50*). In each xylem-bundle the protoxylem lies away from, and the metaxylem towards, the center of the stele (Diagram A). Entering the transition-region the protoxylem broadens and finally divides into two masses which continue to separate as they pass upward (Diagrams B and C). With the separation of the two protoxylem groups the metaxylem is drawn out between them into a broad plate (Diagram D), and in this condition the xylem-mass passes out to the cotyledon (*figs. 55, 56, 61*). In the petiole the metaxylem plate divides, each portion continuing to rotate about its contiguous protoxylem until it reaches the position shown in Diagram E. As the xylem-bundles pass on into the blade of the cotyledon they continue to separate. The metaxylem broadens out, appearing fan-shaped in section (*fig. 58*), and in the center of the cotyledon it nearly surrounds the protoxylem, thus forming a mesarch bundle (*fig. 59*) as described by Worsdell ('97). With the first splitting of the protoxylem-bundle, as it enters the transition-region, a protophloem-group appears between and beyond its two points (Diagram B). This protophloem-bundle increases in bulk as it passes upward, and enters the cotyledon as a single bundle (Diagram D and E). Later it divides to contribute a protophloem-group to each bundle of the cotyledon (*fig. 59*).

In the lower portion of the transition-region, additional protoxylem-elements appear at various points between the cotyledon-traces, and just inside the prospective cambium-zone. Farther up, these scattered elements give place to usually six rather definite strands, which continue upward as the protoxylem-groups of the six primary bundles of the stem (Diagrams B to D and

figs. 52–57). Usually, the four secondary protoxylem-bundles, adjacent to those of the cotyledon-traces, contribute to the latter small groups of elements which unite with them just before they pass out to the cotyledons (Diagram C. and *figs. 55–61*).

While the plan of transition outlined above is often followed to a nicety in a seedling, exceptions are very numerous. The length of the transition-region varies considerably in different seedlings. As a rule, the cotyledon-traces do not leave the stele at the same height (*figs. 51, 61*), and the reduction of the traces into the two root-bundles may take place at very different levels. It is often impossible to recognize distinct bundles in the traces through the absence of protoxylem, or the indiscriminate mingling of protoxylem and metaxylem elements. The sharp limitations of protoxylem and metaxylem indicated in the diagrams are rarely met with in a series of sections.

The origin of the stem-bundles in the transition-region, is usually after the manner already described (*figs. 50–57, 60, 61*). Occasionally, seedlings are met with, however, in which the traces of the first two leaves are prominent in the transition-region, and, with the four bundles of the cotyledon-traces, form a hexarch stele, which may become resolved, lower down, into such a tetrarch stele as seen in fig. 63. Such leaf-traces are often distinctly mesarch. Then again, the trace of a single leaf may continue prominent through the transition-region, and produce a triarch stele for a considerable distance in the root. In a tricotyledonous seedling, the primary root is persistently triarch. Secondary roots are always diarch (*fig. 62*).

Secondary thickening in the stem and root of *Ginkgo* takes place in the ordinary manner. The position of the cambium in the root is shown in the diagrams and in figs. 50–57, 60–64. Below the transition-region, the pith of the root may be partially or almost wholly replaced by metaxylem (*figs. 62–64*). The centrifugal xylem becomes continuous in places with the metaxylem, but it rarely if ever comes in contact with the protoxylem. It is often separated from the latter by only a single layer of pith-cells (*fig. 62*). The growing-point of the root (*fig. 65*) presents essentially the same structure as those of the cycads and conifers which have been described by De Bary ('84). The cork-cambium of the stem arises directly beneath the dermatogen; in the root its origin is deeper seated.

The structure of the cotyledons of the seedling is illustrated

in figs. 58 and 59. The tissues of the petiole are firm, while the portion within the seed is fleshy. The cells of the region which comes in contact with the tissue of the gametophyte are densely packed with starch (*fig. 59*). The rest have fluid contents. Stomata occur on both surfaces of the cotyledon.

Summary.

The essential features of the embryogeny of *Ginkgo* can be summarized as follows.

1. By free-cell-formation, following free-nuclear-division, a spherical protocorm is organized which completely fills the venter of the archegonium.

2. The basal cells of the protocorm, through continued activity, pass over into the blastema or metacormal bud.

3. The meristems of the stem and root are localized out of the one general meristem of the blastema.

4. Cotyledons and leaves arise as exogenous outgrowths upon the growing-point of the stem and are here morphologically homologous structures.

5. Cases are infrequently met with, where two embryos from different oösperms have developed to maturity in the same seed.

6. Polyembryony occurs, occasionally, through the production of two blastemata by one protocorm.

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Description of Plates.

Plates 35, 36, 37 and 39 are after photographs by Mr. C. J. Hibbard.

Plate 29.

1. Sectional view of a protocorm in which free-nuclear-division has ceased. From a section 20 thick (× 160).

2. Section of protocorm just after completion of free-cell-formation. Section 10 thick (× 160).

3. Surface view of a protocorm (× 160).

4. Section of a protocorm in the basal portion of which a blastema is being organized (× 160).

The outlines for figs. 1, 2 and 4 were traced from photomicrographs.

Plate 30.

5. Longitudinal section of a young embryo in which the position of the root-apex is just distinguishable (× 130).

Plate 31.

6. A similar section of an older embryo showing first indication of cotyledon-primordia (× 130).

Plate 32.

7. Shows relation of metacorm to protocorm. The cotyledons are well advanced and secretory canals are appearing in both plerome and periblem (× 57).

Plate 33.

8. From a section of a gametophyte containing a young embryo (× 39). To the right can be seen the empty venter of the second archegonium. Disorganization of the tissue of the gametophyte is evident for some distance ahead of the embryo.

9. An instance where two blastemata were organized in one protocorm, but only one developed into a metacorm (× 36).

10. Section of a young embryo in a plane parallel to the faces of the cotyledons, passing through the stem-apex and primordia of the first two plumular leaves (× 57).

11, 12. Two sections of mature embryos cut, the one parallel, the other perpendicular to the plane of contact of the cotyledons (× 12). These sections show, in a general way, the relation of parts, differ-

entiation of tissues and shape of meristems. The secretory canals are black. The openings in the cortex are resin-reservoirs.

PLATE 34.

13–17. Sections taken at intervals from a series of cross-sections of a dicotyledonous embryo (× 28). 13, through root-meristem; 14, through transition-region; 15, through bases of cotyledons; 16, through cotyledons and stem of plumule; 17, through cotyledons and plumular leaves.

18, 19. Sections of a tricotyledonous embryo (× 28).

20. From an embryo showing a condition intermediate between a typical dicotyledon and tricotyledon (× 28).

21. A section near the tips of the cotyledons of a tricotyledonous embryo, two cotyledons of which developed conjointly (× 28).

PLATE 35.

22–29. Embryos removed from seeds grown in Japan (× 5.5). The dark knobs, very prominent on some of the embryos, are protruding resin-reservoirs.

PLATE 36.

30–37. Embryos removed from seeds grown in the Missouri Botanical Garden (× 5.5). Figs. 36 and 37 are cases of true twinning; two metacorms having developed from one protocorm in each case.

PLATE 37.

38–46. Young *Ginkgo* seedlings about natural size.

PLATE 38.

47. A young seedling which has just completed its growth in length for the first season, slightly reduced.

48. A seedling at the end of its first season's growth, somewhat reduced.

PLATE 39.

49. A young plant early in its second year, somewhat reduced.

PLATES 40 AND 41.

50–57. Sections, selected at intervals from a series, passing from the root through the transition-region to the stem of a young seedling (× 92).

PLATE 42.

58, 59. Sections of a cotyledon of the seedling shown in Fig. 48 (× 28). Fig. 58 is through the petiole, and Fig. 59 through the thickest portion of the cotyledon. The circular openings in Fig. 58 are resin-reservoirs.

60, 61. Sections through the transition-region of a young seedling (× 60).

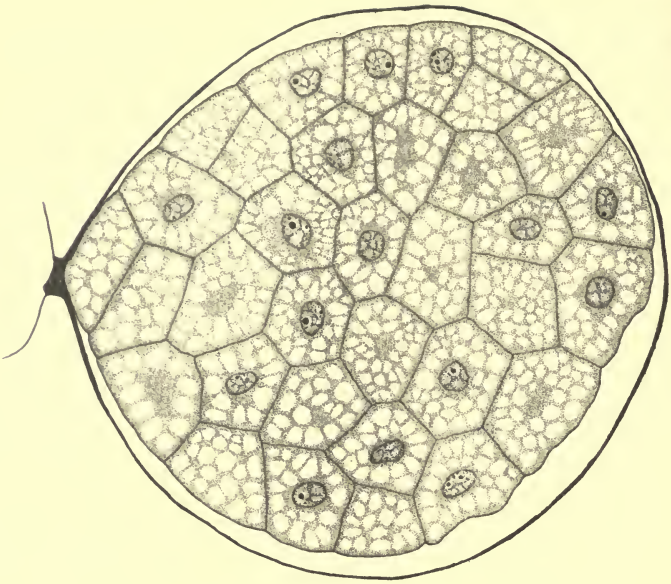
PLATE 43.

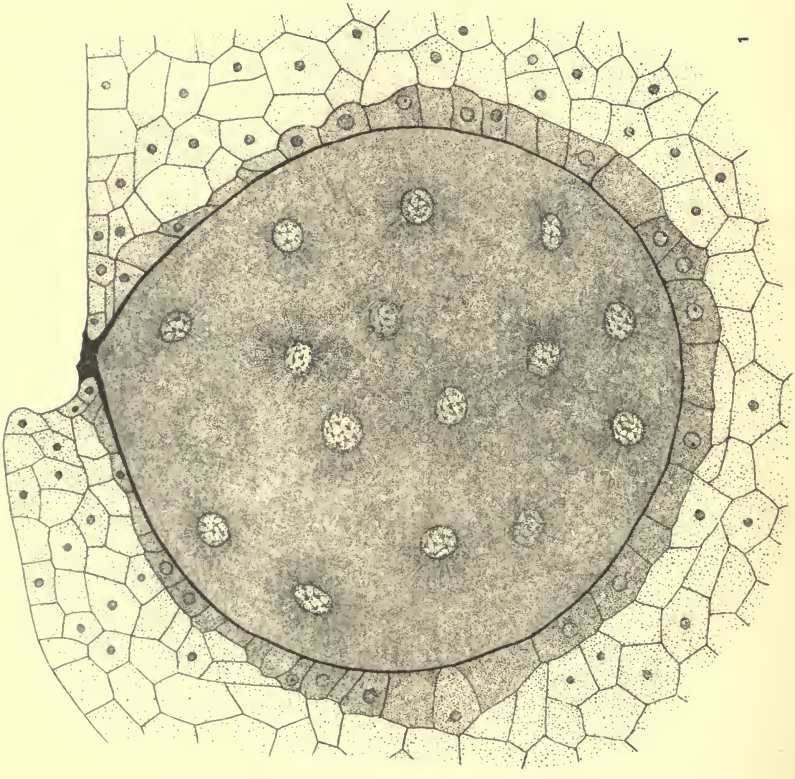
62. Cross section of a secondary root (× 62).

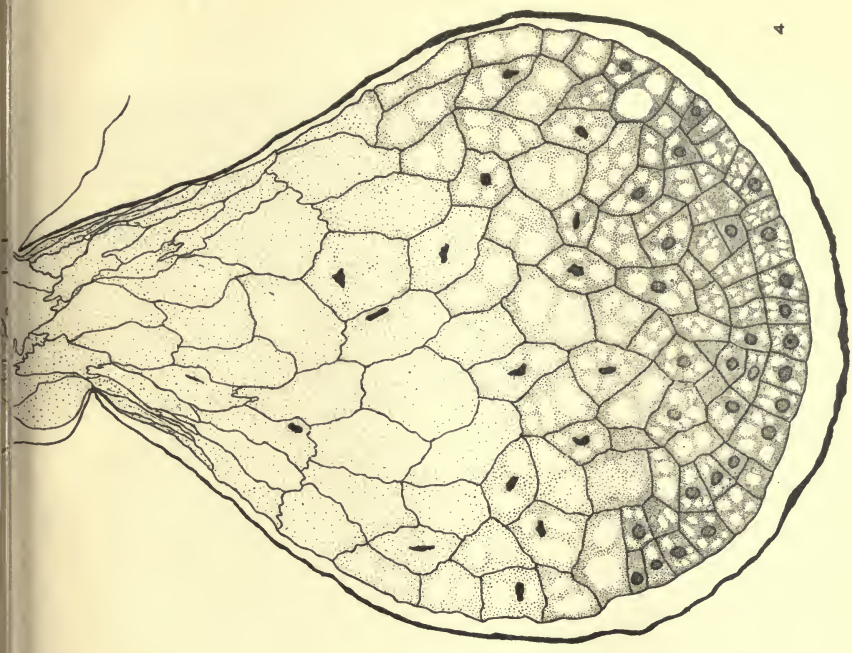
63. Cross section of a primary root in which the traces of the third and fourth leaves continued distinct through the transition-region, and at the point of this section, formed a tetrarch arrangement with the two cotyledon-traces (× 57).

64. Cross section of a primary root in the lower portion of the transition-region (× 25). A central mass of pith is completely surrounded by metaxylem.

65. Longitudinal section through the tip of a secondary root (× 110). A flake of tissue, torn from the primary root, adhered to the tip of the secondary root and appears in the section.







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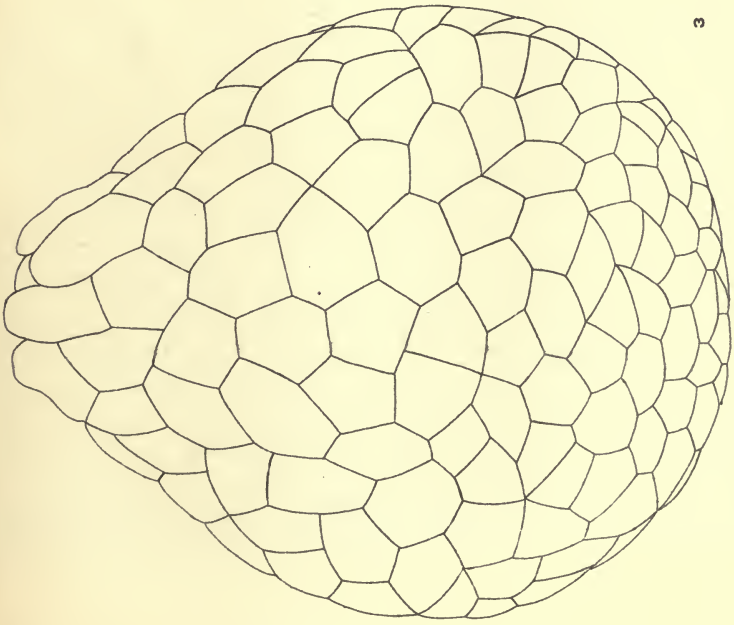

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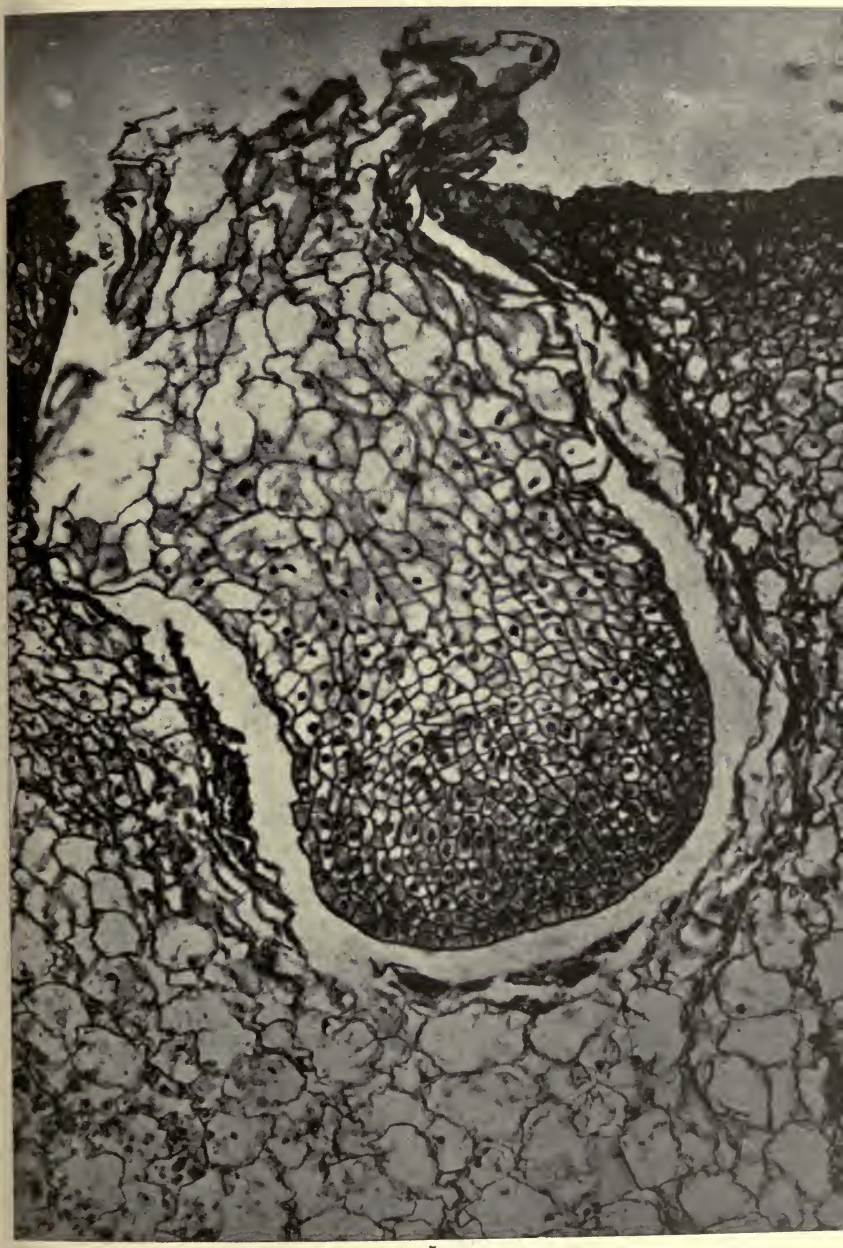

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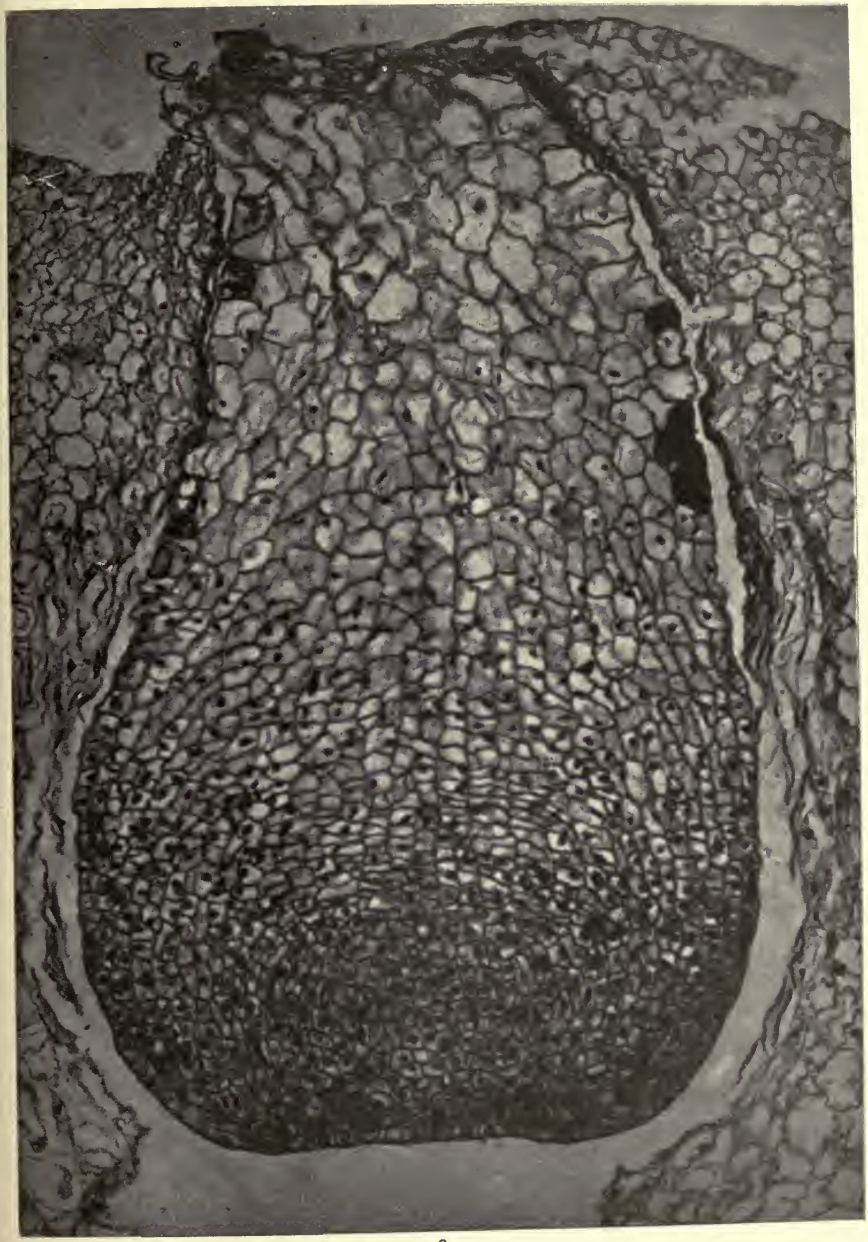

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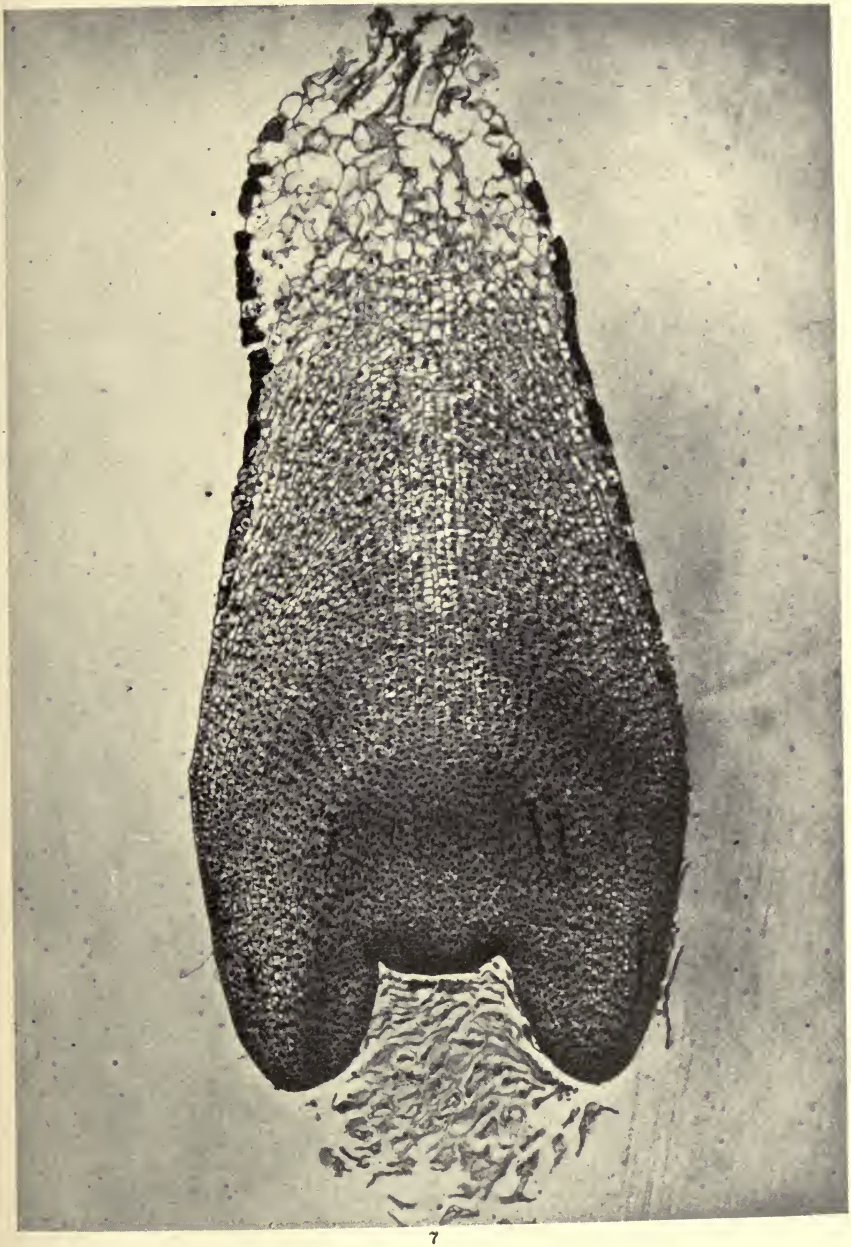

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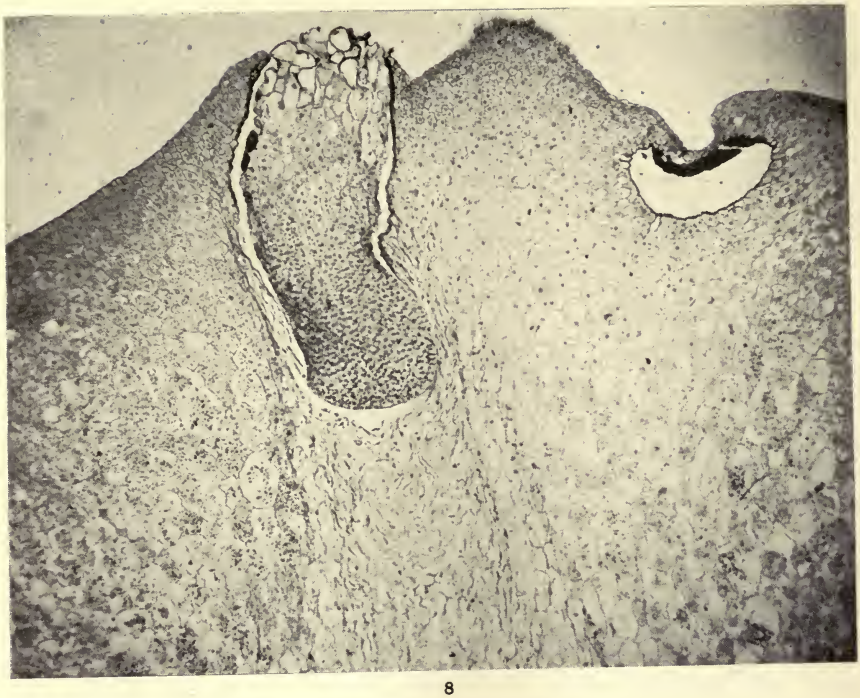

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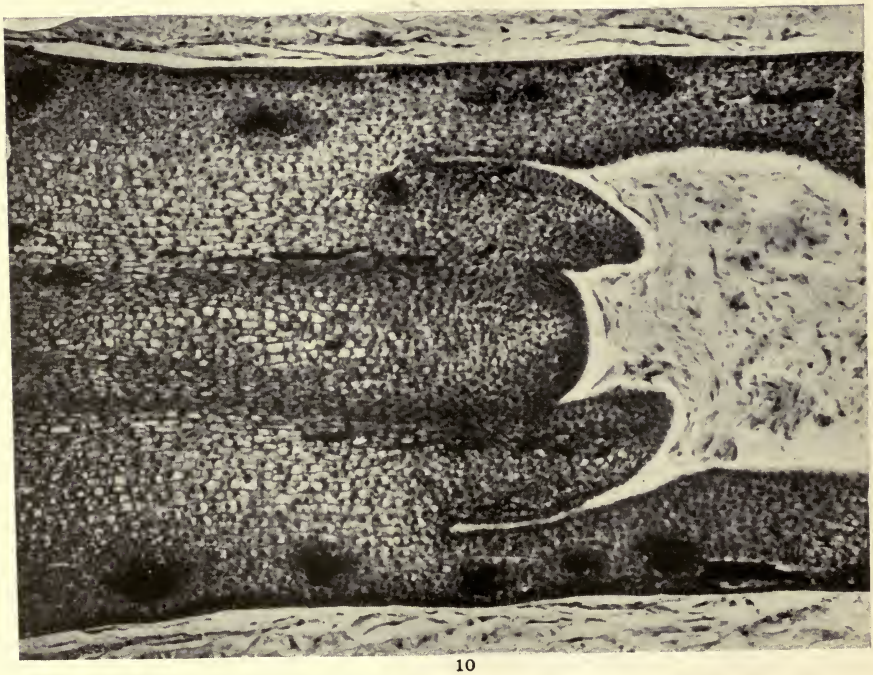

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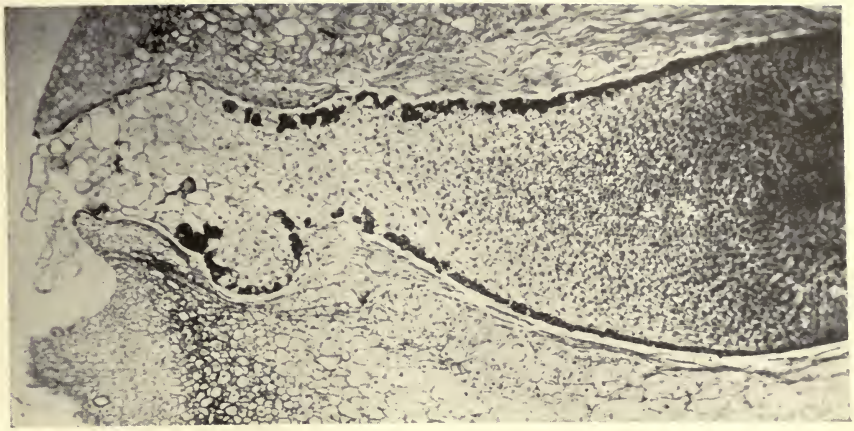

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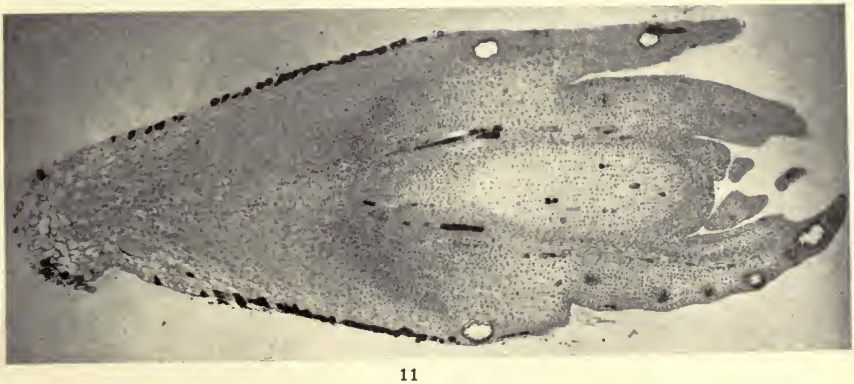

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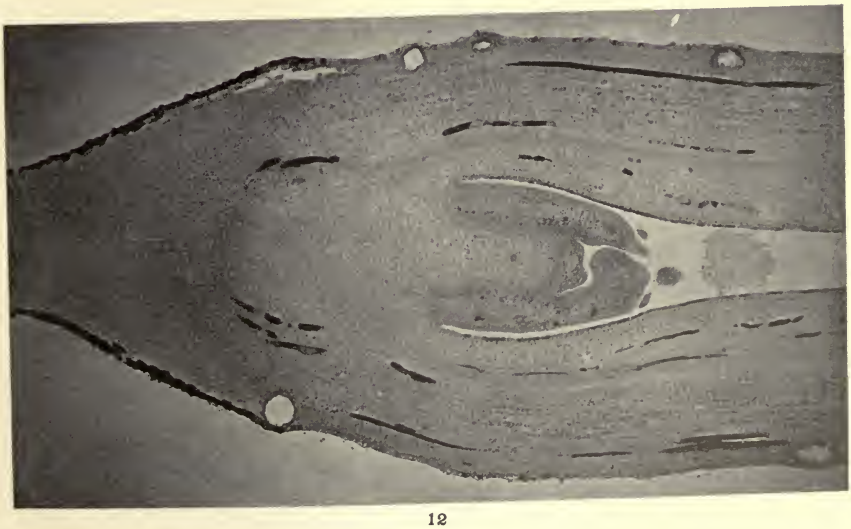

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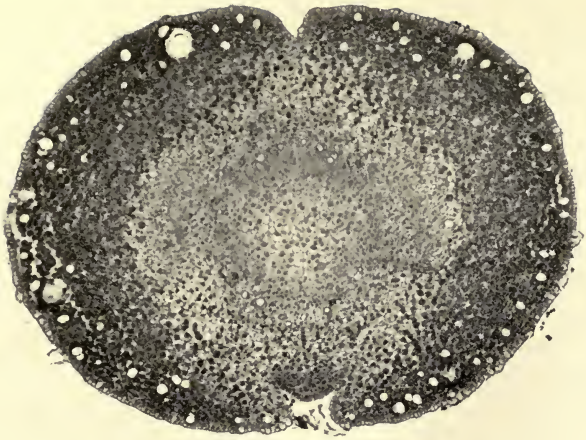

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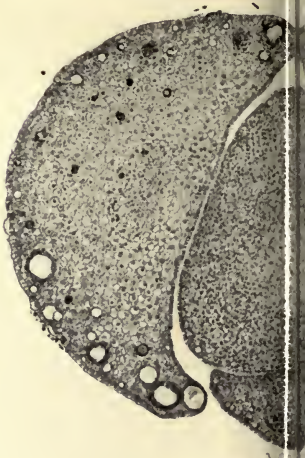

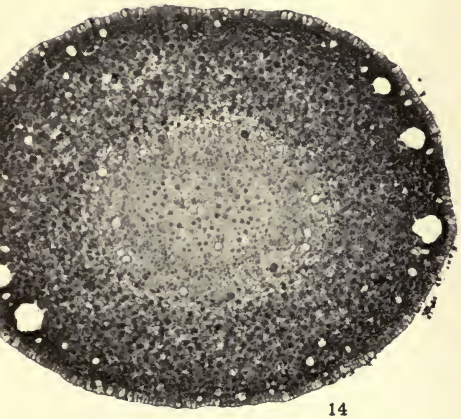

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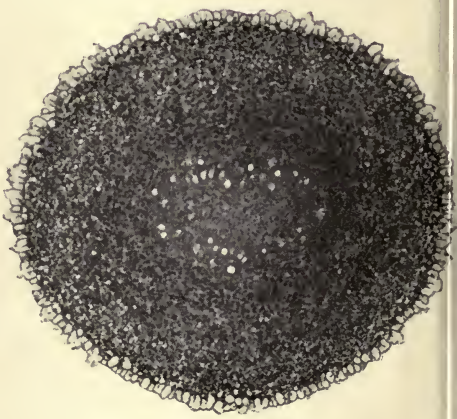

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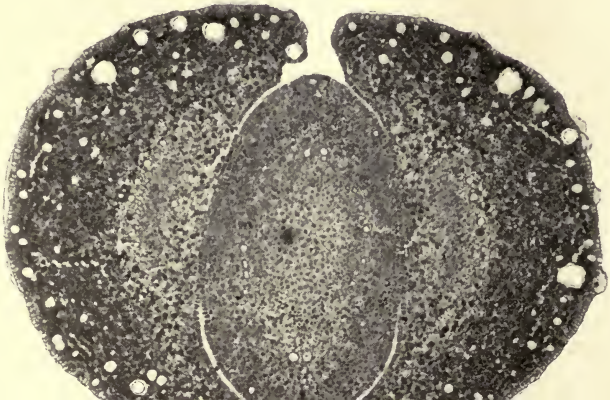

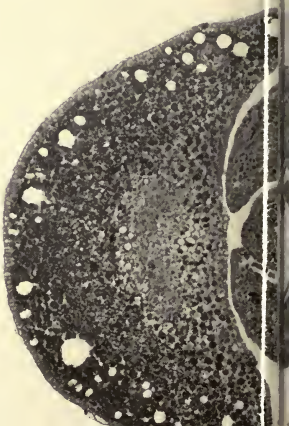

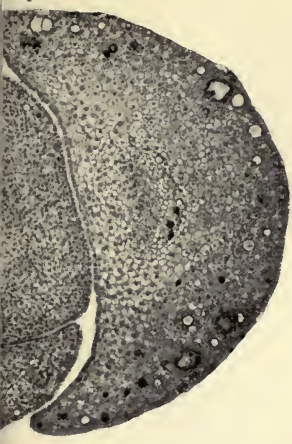

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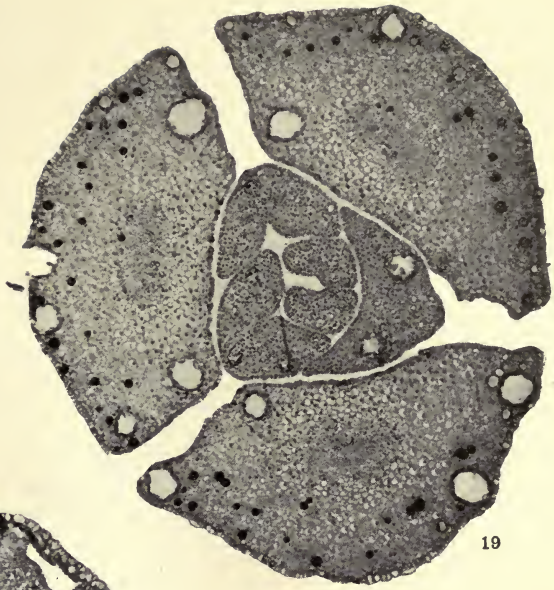

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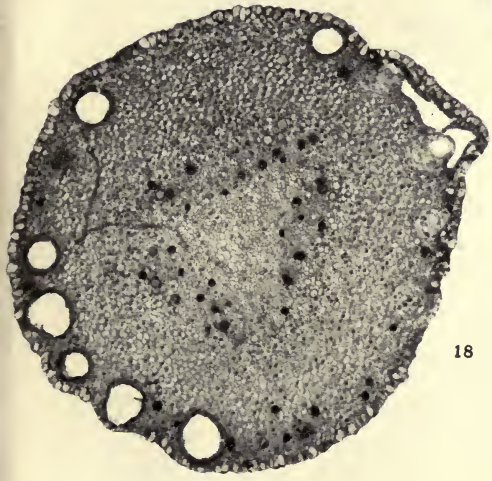

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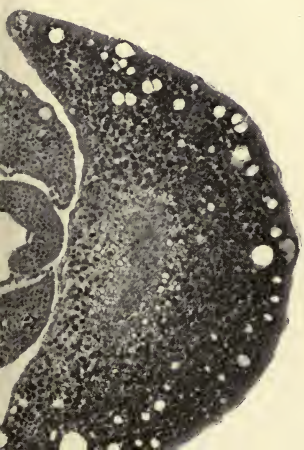

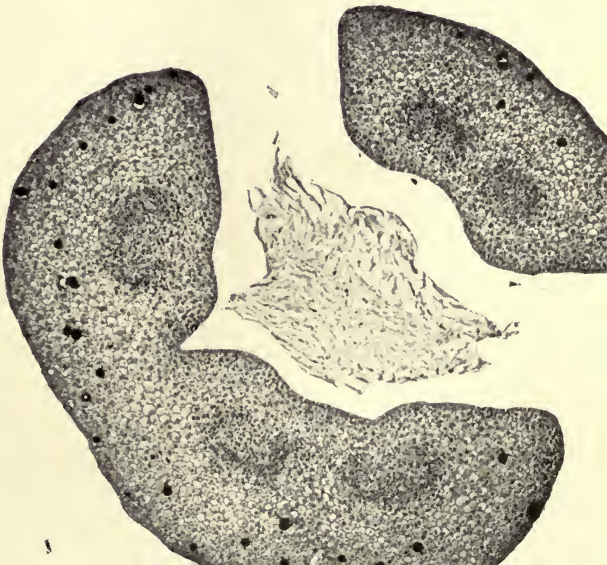


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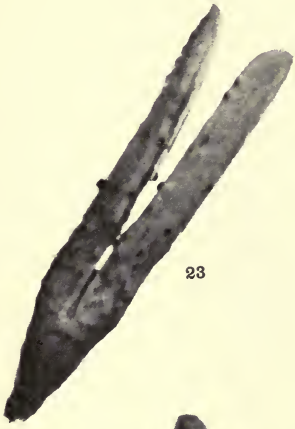
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PLATE XXXV.


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PLATE XXXVI.

PLATE XXXVII.

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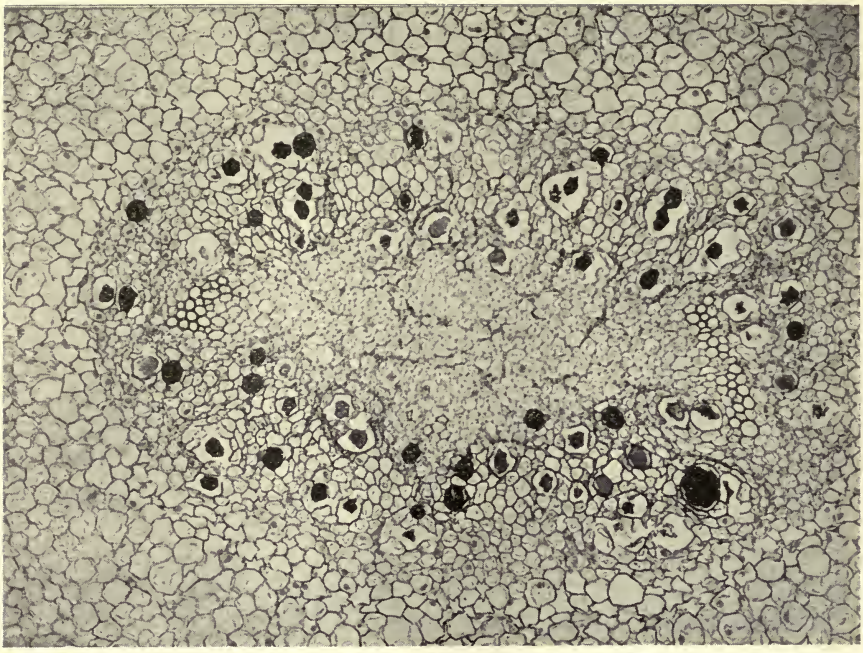
PLATE XXXVIII.

PLATE XXXIX.



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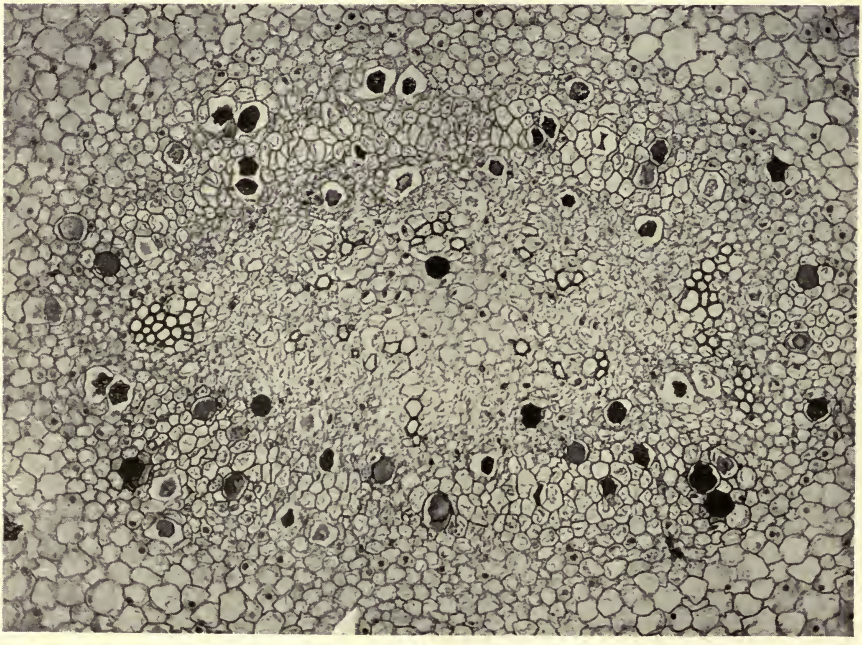

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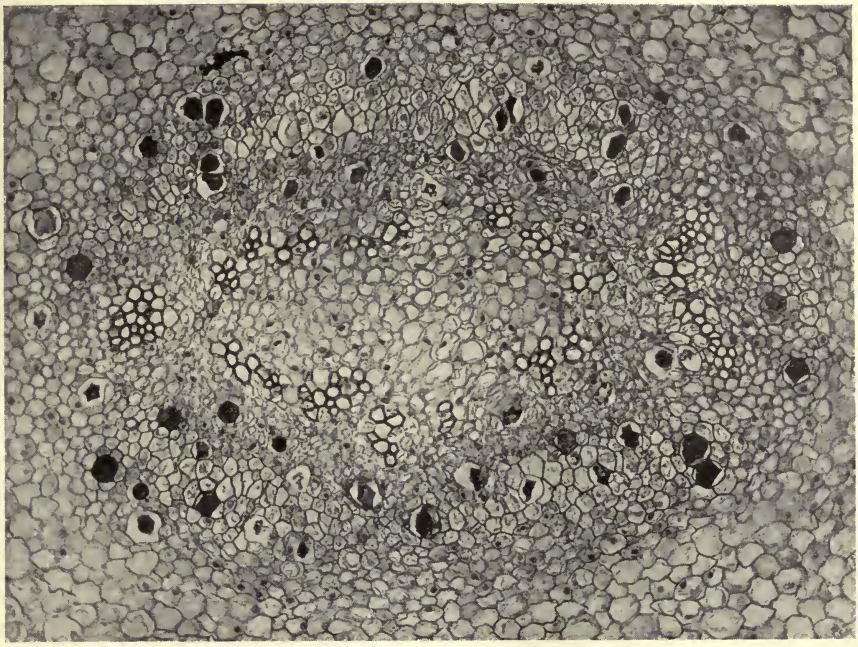

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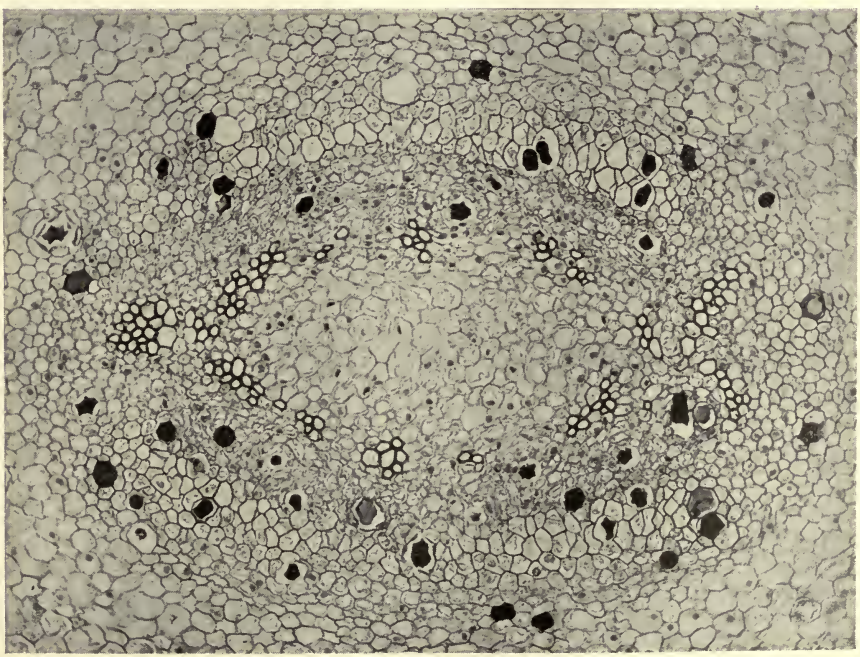

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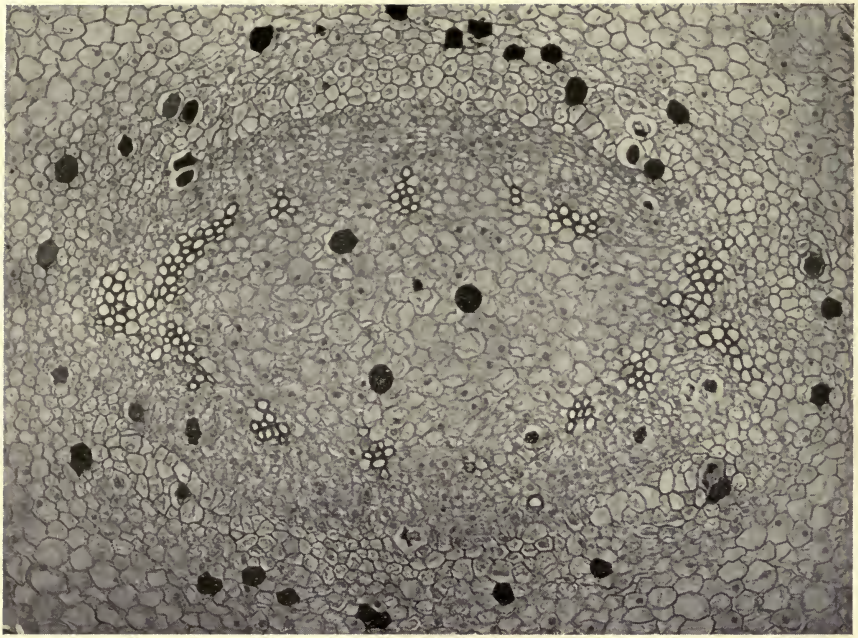

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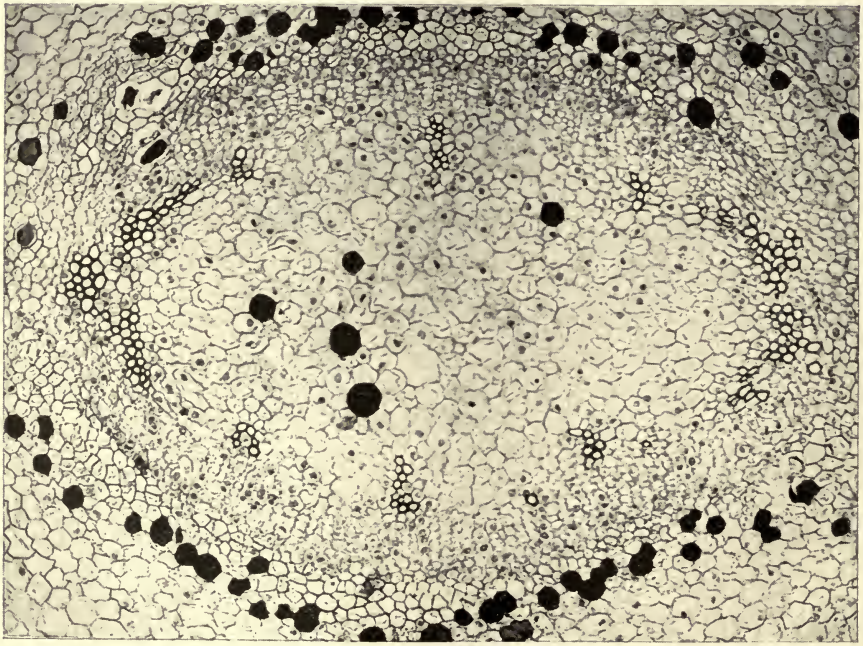

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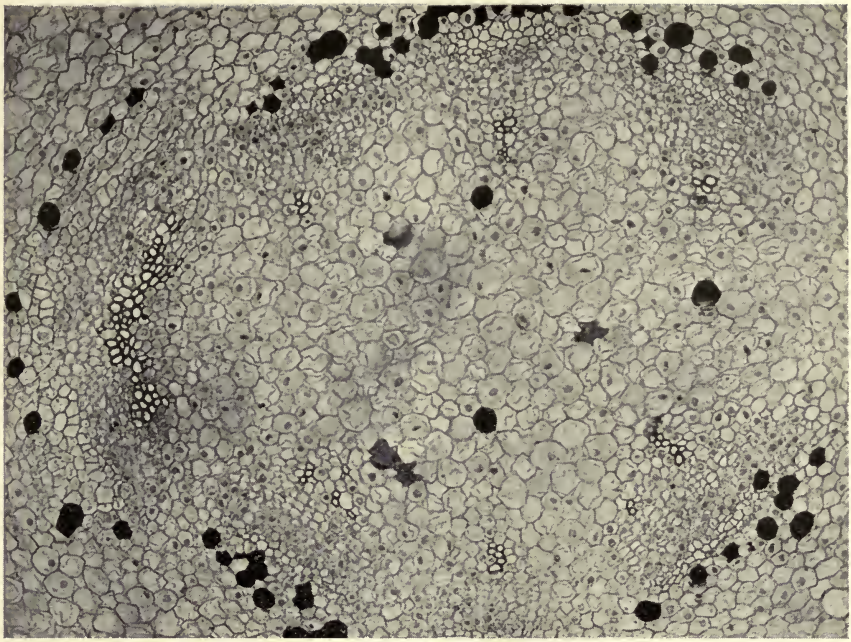

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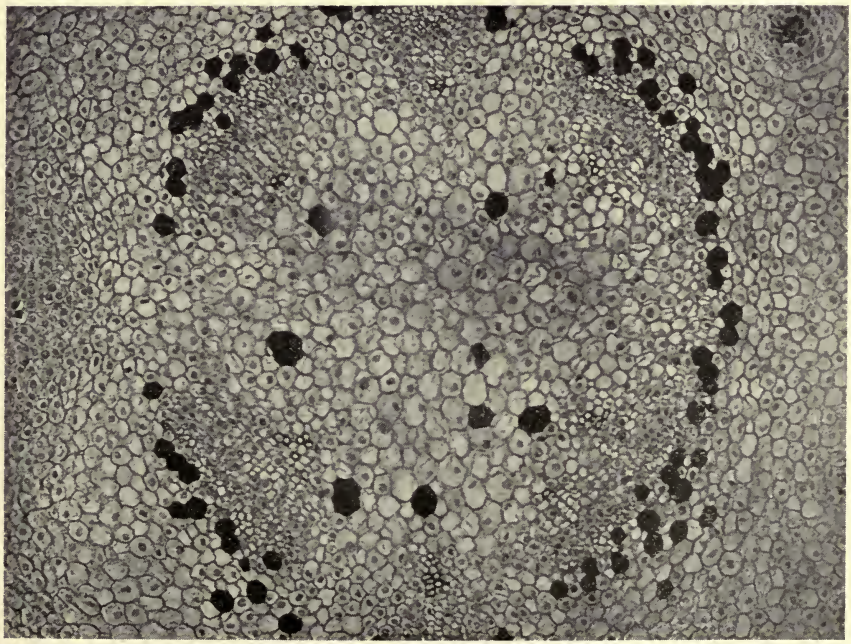

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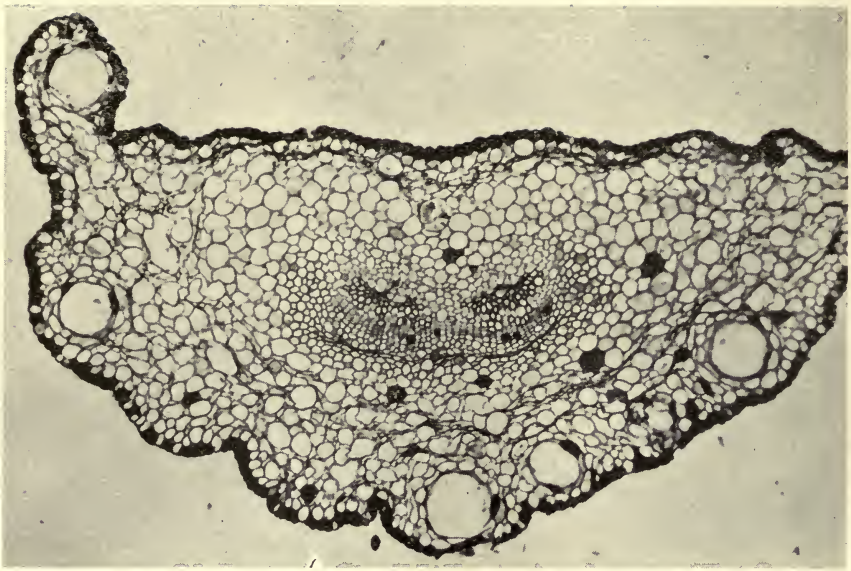

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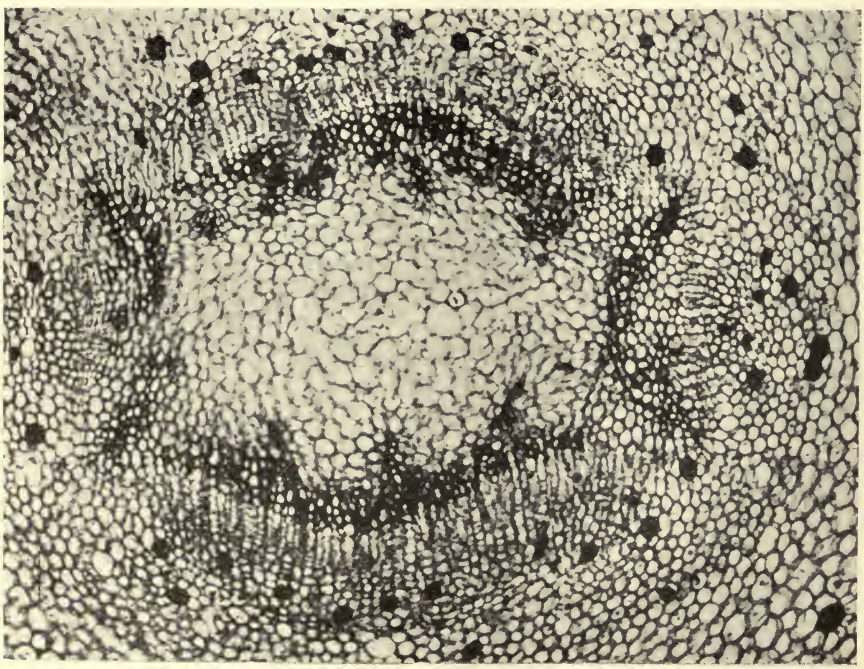

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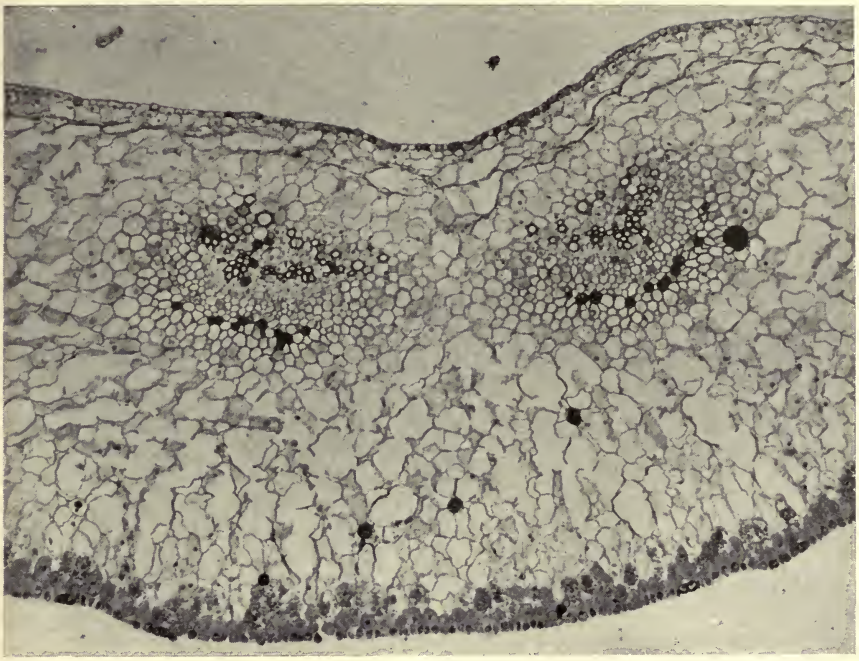

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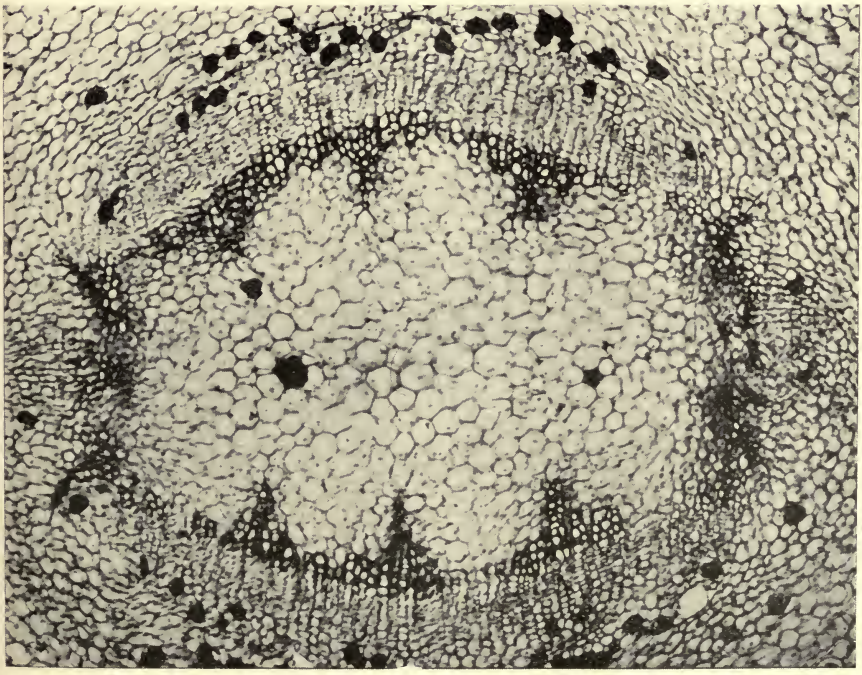

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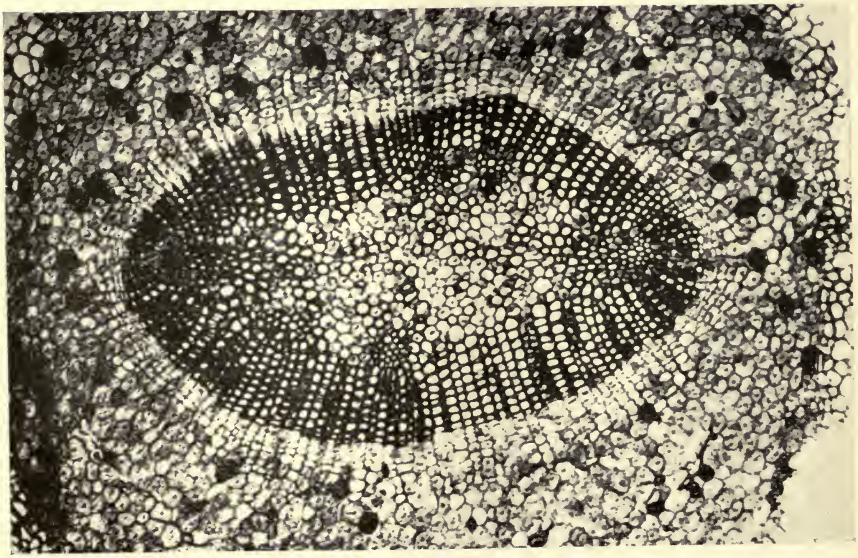

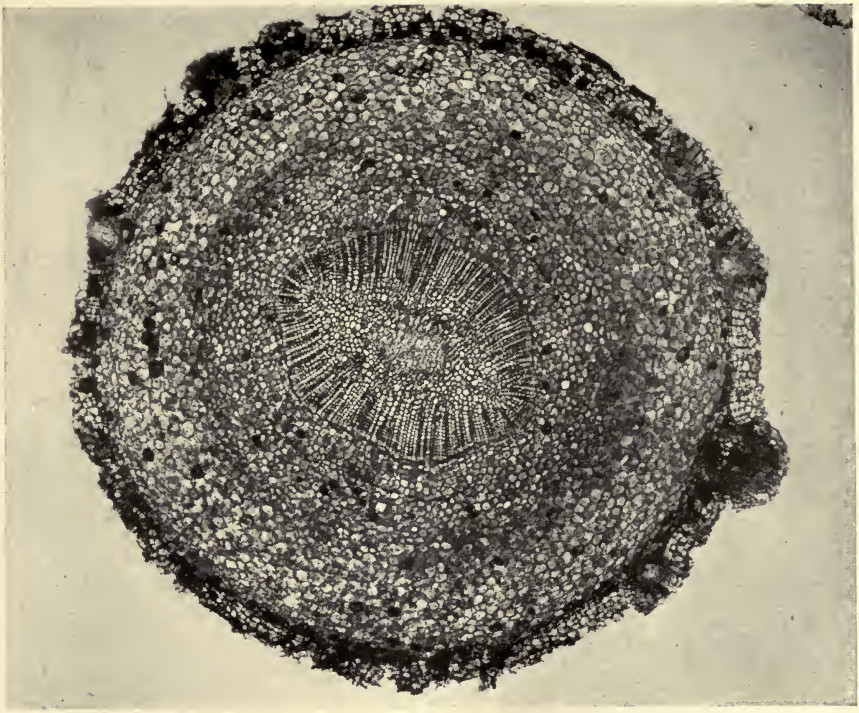

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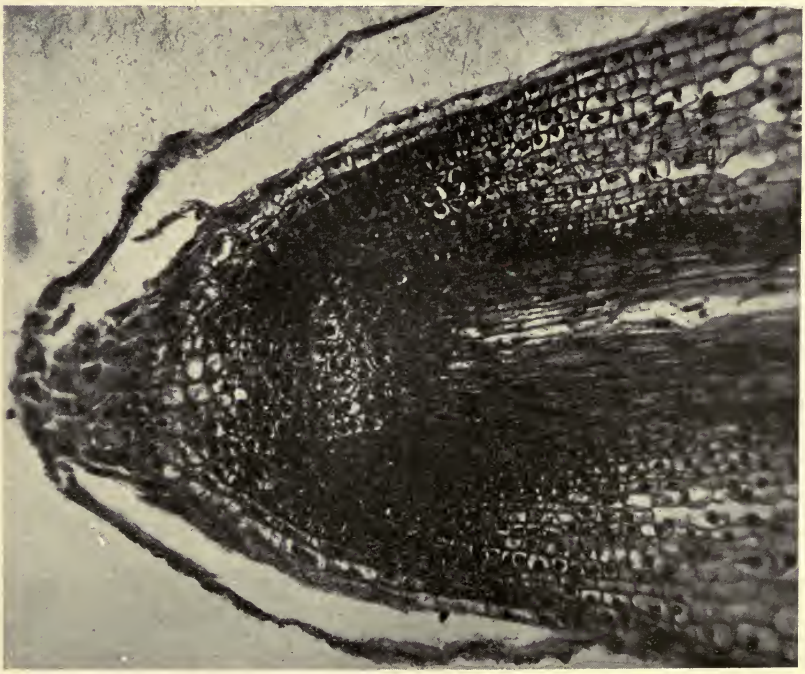

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XXIV. OBSERVATIONS ON CALLYMENIA PHYLLOPHORA J. AG.

Clara K. Leavitt.

Material.

This study was made from plants collected by the writer in July and August, 1901 and 1902, and by Miss J. E. Tilden in December, 1901 at the Minnesota Seaside Station, Port Renfrew, B. C. All the older plants were washed up on shore. Only very small young plants were collected on the rocks at low tide.

Habitat.

The plant is elittoral. It occurs in crevices in the rocky caverns where the surge is strong as the tide runs in and out. It grows in large quantities on the rocks running out from Johnson's Cove and is always to be found in the wash in the cove. It is also attached to rocks on the sea bottom and was sometimes washed in attached to the holdfast of a *Nereocystis*. Only young plants were uncovered by the low tides (−1.2 ft.) of August 2–4, 1902. The mature plants were well out beyond the low tide line, indicating that the conditions were more favorable at the lower points.

Gross Structure.

The young plant consists of a small disk-shaped holdfast, a short cylindrical stipe and one or sometimes two, entire somewhat circular laminæ (*fig.* 1). As the plant grows older proliferations appear on the margin of the lamina, each consisting of a short cylindrical stipe and a broad, flat, usually oval blade. The main central lamina later becomes thickened longitudinally from the secondary stipes on the margin to the main stipe, forming a sort of palmate veining (*fig.* 2). A tearing of the lamina between the "veins" results, and the stipe appears to have several laminæ arising from it, where originally there was but one. Often several laminæ arise from one holdfast each on a distinct stipe. The older primary fronds are dark red in color, thick, coarse and leathery, while the younger laminæ are much

finer and thinner and of the same dark red color. The secondary laminæ not infrequently arise from the surface instead of the margin of the primary frond (*fig. 2*). A third series of laminæ rising from the margin of the secondary laminæ was common, but a series of four as in fig. 3 was comparatively rare. A plant often reaches twenty-five and thirty centimeters from stipe to margin of outermost lamina. The dried specimen does not adhere well to the paper.

Minute Structure.

The material used consisted chiefly of free-hand razor sections of fresh plants gathered in the summer of 1902. The mature cystocarps were studied from formalin material collected in December, 1901, at the same point.

1. *Holdfast.* — The holdfast is comparatively small and fits like a sucker to the surface of a flat rock or curves over a projection or barnacle (*figs. 4, 5*). A thick layer of gelatine lying close to the rock fits into all uneven places and takes the impression of the rock (*fig. 6*). Above this, thick at the center and thinner at the margin, lies a layer of cortical cells covered by an epidermal layer several cells in thickness (*fig. 7*). Several holdfasts were sectioned in which a second cortical and epidermal layer appeared to have grown out over the first, so that a layer of gelatine lay within the tissues of the holdfast.

2. *Stipe.* — The main stipe was about five millimeters in length, cylindrical in form, becoming oval in cross section where it passes into the lamina. The older stipes are thicker but not longer than the younger ones.

The epidermis consists of deeply colored, thick walled, somewhat rectangular cells arranged in several rows. The cells of the true epidermis are rather larger than those of the subepidermis. Dimensions, 6.5 to 16.5 mic. in length and 6.5 mic. in width (*Plate XLIV., fig. 8*).

The cortex is made up of large, clear, colorless cells. The cells of the inner cortex are much larger than those of any other part of the plant. Dimensions, diameter 16 to 50 mic. (*Plate XLIV., fig. 9*).

In the center lie the long, narrow, thick-walled cells of the pith strand. These run mainly longitudinally, very few running transversely. Dimensions, diameter 20 mic. (*Plate XLIV., figs. 10, 11*).

Peculiar layers of tissue appeared in some of the older stipes. These remind one of annual rings. Similar structures are reported by Jonsson in such related forms as *Ahnfeltia plicata* and *Phyllophora membranifolia*. In these Jonsson found a very clear layering of the cortex marked by a difference in color and diameter of cells and formed probably by a division of the cells of the outer cortical layer.

The layers of stipe in *Callymenia phyllophora* as observed in the few older stipes collected, showed no such origin. They are irregular as to width and position, sometimes encircling the stipe, sometimes appearing on one side only. Some partial annulations were apparently due to a change of direction of the filaments of the medullary layer. Most of the filaments of one layer ran in a direction perpendicular to those of the next layer.

In other instances the annulations were apparently due to an overgrowth covering a primary cortical layer. The epidermis of the inclosed layer was somewhat disorganized and the gelatine was filled with diatoms which thus became imbedded some distance in the stem. Several layers of cortex and epidermis, distinctly marked by their imbedded parasites, appeared on one side of some stipes. In these instances the buried epidermal cells were very distinct.

Lamina.

The lamina is made up of the same three tissues, an epidermis from three to five cells in thickness, a cortex two or three cells deep and a pith strand occupying the main cross section (*figs. 12, 13*).

The leaf is abruptly thickened at the margin, due mainly to the greater number of cells in the pith strand and cortical layer (*fig. 14*).

Fruit.

The cystocarps form dark dots showing through the surface of the thallus when held to the light. The mature cystocarp increases only slightly the thickness of the lamina in the center of which it lies. The spores are of rounded but rather irregular form, many of them enclosed together in a compartment-like portion of the cystocarp formed by a single row of long clear cells (*fig. 15*). The cystocarp had no well defined wall as described by Carruthers for *Rhodymenia*. The spores are discharged by a rupture of the epidermal layers of the lamina just

over the cystocarp. Dimensions of spore, length 20 to 35 mic. width 13 to 20 mic.

Young cystocarp material was collected late August, 1902, showing the oögonium and accessory cells in figs. 16 and 17.

The stages of development described in *Callymenia* J. Ag. by Bornet could not be made out.

Parasites.

Microcladia coulteri. — This plant occurred parasitic on nearly every plant collected. The largest found was six centimeters in length. It is usually found on the margins of the fronds but sometimes occurs on the surface. A section through the lamina of the host shows the rounded base of the parasite standing in a cup-like depression of the host thallus formed by the disappearance of the epidermal cells. Long rhizoid-like cells project from the short rounded cells at the base of the parasite down between the cortical cells of the host (*Plate XLV., fig.* 1).

Callithamnion sp. — Almost all laminae showed at some point traces of this parasite. The primary frond is frequently covered with a fine short downy mat of it. Plate XLV., fig. 2, shows a young filament extending up from the surface of the host thallus. Beneath the surface the parasite sends down a long rhizoid to the pith strand of the host through which it ramifies. This rhizoid sometimes branches in the pith strand. Above the surface the *Callithamnion* shows its characteristic branching. Plate XLV., fig. 3 shows this parasite in the tetragonidial condition.

Comparison of Microcladia and Callithamnion.—The former is comparatively large, is borne on the margin of the thallus and its penetration into the pith strand was slight, not branching in any direction. The latter was either invisible to the naked eye or appeared as minute down on both surfaces of the host thallus. It penetrated through all parts of the host as a single branching filament.

Porphyra sp. — Young *Porphyra* plants were found growing epiphytically upon this parasitic *Callithamnion*. Plate XLV., fig. 4, shows such a plant consisting of a filament of four cells which broadens out to form the characteristic flat thallus of Porphyra. At the base the filament forks, forming two branches of three cells each, which served as a holdfast.

Chlorochytrium inclusum. — A section cut in fresh material August 12, 1902, showed a mature vegetative stage of this parasite, Plate XLV., fig. 5. It corresponded very closely to the parasite on *Constantinea rosa marina* found and described by Mr. E. M. Freeman. Dimensions: 150 mic. × 75 mic.

It was oval in form and was inclosed in a thick cell wall which protruded beyond the epidermis of the host. The parasite lay mainly in the epidermis scarcely extending into the cortex of the host. Several distinct pyrenoids could be distinguished in the bright green chlorophyll.

Endophyte, genus unknown. — This parasite was to be found in the great majority of the sections cut in the laminæ. It occurs but rarely on stipe or holdfast. It is usually distinctly vase shaped with the interior of the vase toward the interior of the lamina. It often lies entirely in the subepidermis and never extends beyond the surface of the host. The larger ones extend down into the outer cortex. Each is enclosed in a thick wall. The contents are homogenous, granular and vary from an almost colorless to a yellow green in color. Often what appears to be a large oil drop lies in the base of the vase. The parasite consists often of two and sometimes of three or four cells (Plate XLV., fig. 6), one large cell in the base of the vase and one or two in the narrow neck. The larger plants are often one celled, Plate XLV., fig. 7, and the small ones are sometimes two.

Dimensions: length 35 mic. to 140 mic. width 25 to 45 mic. Plate XVL., fig. 8, shows diagramatically the relative positions of the cystocarp and the two parasites *Callithamnion* and ——.

Explanation of Plates.

Plate XLIV. figures *Callemenia phyllophora f. orbicularis* as to general habit and structure. Plate XLV. figures various parasites upon the plant.

Plate XLIV.

1. A young plant about half natural size.
2. Older plant with secondary laminæ rising from margin and surface, about one half natural size.
3. Mature plant consisting of series of four laminæ, about one half natural size.
4. Joint holdfast of young and mature frond attached to clam shell, about natural size.
5. Side view holdfast, natural size.
6. Sucker-like under surface of holdfast, natural size.

7. Cross section of epidermis and cortex of holdfast, × 250.
8. Cross section through epidermis and cortex of stipe, × 250.
9. Detail of cortex cells of stipe, × 250.
10. Cross section through pith strand of stipe, × 250.
11. Longitudinal section through pith strand of stipe, × 250.
12. Cross section through epidermis of lamina, × 250.
13. Cross section through cortex and pith strand of lamina, × 250.
14. Diagram of cross section of leaf showing thickening of margin due to increase of cortex and pith strand, × 30.
15. Section of cystocarp showing spores inclosed by row of clear cells, × 250.
16. Oögonium and accessory cells among cortical cells of lamina × 250.
17. Another oögonium and accessory cell, × 250.

Plate XLV.

1. Outline of young thallus of *Microcladia coulteri* from the base of which project long rhizoid-like cells into the cortex of the host plant, × 170.
2. Young filament of *Callithamnion* among cells of host, × 170.
3. Filament of *Callithamnion* bearing tetragonidia, × 170.
4. Young frond of *Porphyra* epiphytic on parasitic *Callithamnion*, × 170.
5. *Chlorochytrium inclusum* lying in epidermis of host, × 170.
6. Endophytic parasite of unknown genus consisting of four cells, × 170.
7. Same parasite surrounded by epidermal cells, × 170.
8. A diagram of cross section of lamina showing relative positions of cystocarp, parasitic *Callithamnion* and endophyte, × 20.


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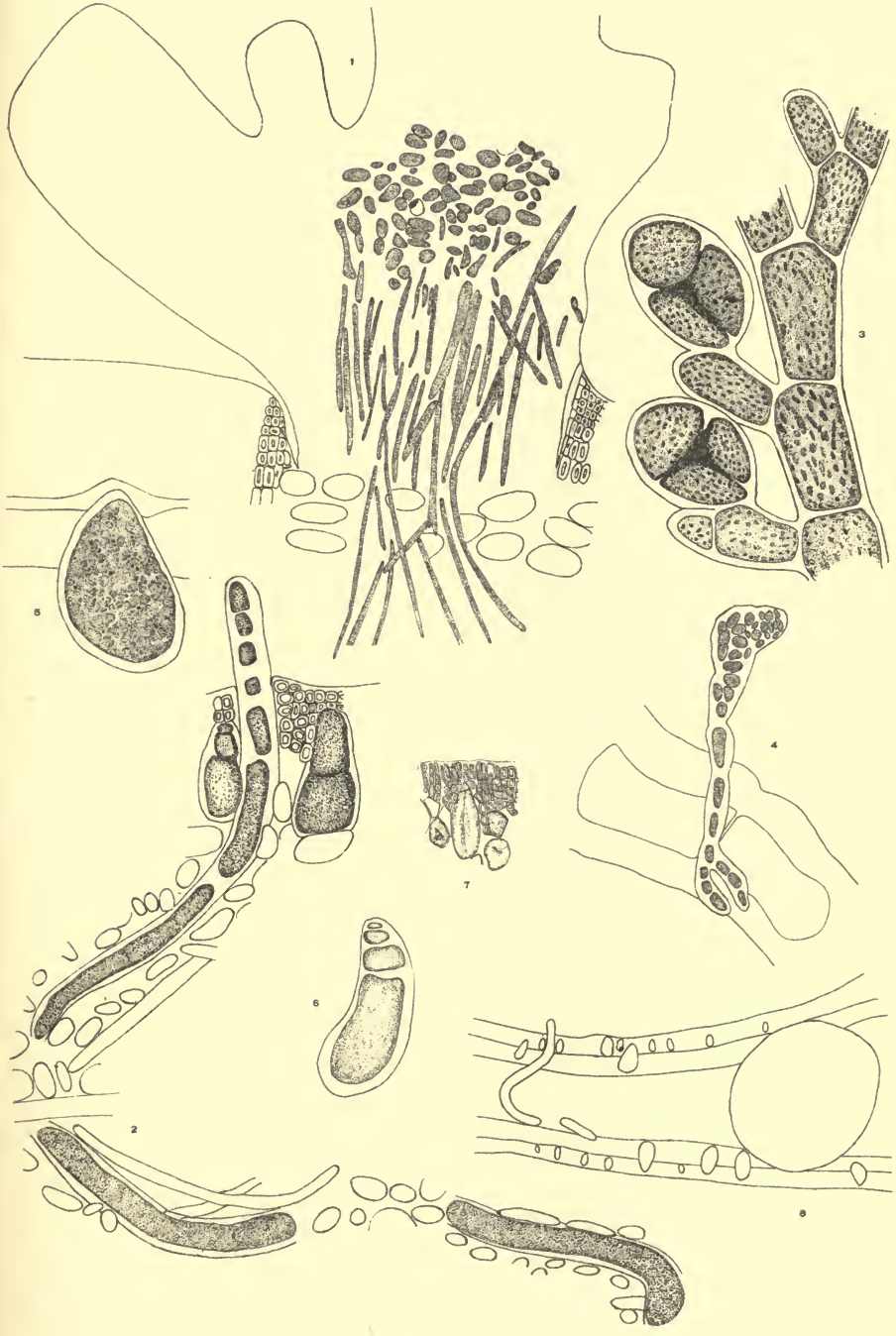
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XXV. OBSERVATIONS ON ENDOCLADIA MURICATA (P. AND R.) J. AG.

FLORENCE M. WARNER.

The collections upon which this paper is based were made at the Minnesota Seaside Station in August, 1902.

This species was given the same name by two different students of algæ at about the same time. Harvey gave the name *Gigartina muricata* to a form from San Francisco in 1839 or early in 1840 while Postels and Ruprecht gave the same name to a form of the same species in 1840. Harvey describes the plant (Ner. Bor. Am., p. 182, *pl. 27, B*) as follows: "The frond is formed of a simple, jointed axial filament of large diameter, with internodes containing endochrome and about thrice as long as broad, coated externally by a thin stratum of minute cellules, from which radiate to all sides numerous, dichotomous, moniliform, horizontal filaments, whose apices, strongly soldered together, unite to form the periphery. The substance is firmly cartilaginous, rigid when dry, Color a very dark brown. Conceptacles spherical, sessile on the ramuli."

Schmitz and Hauptfleisch in Engler and Prantl's Die natürlichen Pflanzenfamilien, describe *Endocladia* and figure the three-celled carpogonial branch with the auxiliary cell which form the procarp of *E. vernicata* J. Ag. The thallus, according to the above, is cylindrical, very much branched on all sides with small hooked spines, and has a distinct filamentous structure. There is a rather thick, long-jointed central axis, with an alternating, inclined, jointed apical cell. This sends off in alternate order dichotomously branched filaments which grow diagonally upwards. These branches are more loosely constructed and longer jointed toward the center, but toward the cortex become smaller celled and closer, lying finally side by side. The inner layer is more or less quickly traversed with dichotomously branched short-celled rhizoids. The central axis is surrounded by numerous analogous filaments running lengthwise. Gonidia are found in great numbers in the thickened branches of certain sections of the thallus. Procarps are

found in the somewhat loosened, fruit-bearing section of the thallus appearing in great numbers in the central part of the cortex. A short, two or more celled, many forked, small secondary side-branch of a filament forms an auxiliary cell from an end cell. Near the auxiliary cell is developed a three-celled carpogonial branch bent in shape of a hook. The gonimoblast apparently arises from the fecundated auxiliary cell, branches profusely, at times towards the center, into the somewhat loosened tissue of the inner layer. The branches of the gonimoblast creep between the rows of cells of the sterile tissue, often fusing with these cells, and finally the end cells develop into spores.

The fruit-body is an irregular mass of interlacing fibers of which the lower, stronger sections of the branches stand out plainly, with numerous spores irregularly massed in the interstices. The cystocarp, without a special protective layer, is sunk in the locally, slightly thickened thallus. It protrudes slightly to one side of the thallus near the short spiny point of a branch. The fruit wall formed by the local thickening of the cortex of the thallus does not show a pore.

The same authors describe the reproductive organs of the Gigartinaceæ as follows: Reproduction occurs both sexually and non-sexually. The tetraspores are strewn over the surface under the outer cortex or in many irregular groups and then sunk in the inner cortex of the thallus, or arranged in projecting nemathecia. The sporangia usually divide transversely but they also divide obliquely (*Endocladia*).

Antheridia are spread over the upper surface of the thallus, sometimes in the form of small, cup-shaped capsules, opening outwards, and sunk in the outer cortex of the thallus.

The carpogonial branch develops from a lateral branchlet of a primary branch. The carpogonial branch is three-celled, bent inwards like a hook, and connected with the swollen auxiliary cell, rich in contents. The fecundated auxiliary cell grows inwards and develops the gonimoblast branches. The end cells of these branches are transformed into spores.

Endocladia hamulosa (Ruprecht) J. Ag., described in De-Toni's Sylloge Algarum, seems to differ from *E. muricata* only in the position of the cystocarps. "*E. hamulosa* seems to differ from *E. muricata* only in having the cystocarps at the bases of the ramuli, while in the latter species they are simply

lateral. We have found both sorts on the same plant so it has seemed best to include both under the same name"—Setchell and Gardner. This is true also of the specimens examined by me.

Endocladia muricata (P. and R.) J. Ag. is a red alga belonging to the genus Gigartinaceæ. The plants studied at the Minnesota Seaside Station, Vancouver island, seem to be the typical form. Setchell and Gardner describe two other forms, *E. muricata* forma *compressa* and forma *inermis*, but as the specimens in hand have not a particularly flattened frond and are not destitute of spines, they are probably neither of these forms.

The plants were found growing on rocks and boulders in the upper portion of the littoral zone very near high water mark. They were fastened quite firmly to the substratum. The fronds are low, from 2–4 cm. in height, shrubby in appearance, and very dark red or brown in color. The branching is dense and irregular and the branches are profusely covered with spines. The frond seems to proceed from a branch which runs horizontally along the surface of the substratum. This horizontal branch sends off downward branches at the ends of which holdfasts are developed. Upright branches develop into the frond.

Frond.—Examining a longitudinal section (*Plate XLVI., fig. 4*) of the frond a conspicuous central cylinder is seen surrounded by a mucilaginous sheath. This axis is divided into cells about three times as long as broad. There appear to be protoplasmic connections running through the dividing cell walls of the axis cylinder. Branches are given off quite regularly from this central axis, the branches arising just below the cross walls of the central axis, and often from two sides of these cells. These branching filaments do not extend radially out to the cortex as described by Harvey, but rather diagonally upward and outward, terminating in the cortex opposite the lower part of the third cell of the central axis from which they started (*Plate XLVI., fig. 4*). The branching seems to be more or less regular (*Plate XLVI., fig. 2*). Two branches are given off from the upper third of the cell, following somewhat the method of branching of the central cylinder. The branching, in this way, seems to be quite regular for about eight cells, when it sends off two branches, each of these branching dichotomously until the cortex is reached. There are, however, exceptions to this rule.

Massed around the central axis are small round cells. These seem to have developed, at least in some cases, from the upper branch (*Plate XL VI.*, *figs.* 2, *a*, and 3, *a*) of the original branch coming from the central axis. Schmitz and Hauptfleisch speak of these rounded cells as rhizoids, while Harvey does not discuss their origin, but speaks of them as coating the axis. In the material studied the branching was not always dichotomous nor did the branches run radially outwards. The filaments in the center of the frond are loosely scattered, being massed together closely to form the cortex. Examining a cross section of the frond we find a large round central cylinder (*Plate XL VI.*, *fig.* 1). Massed around this cylinder are small round cells. The cells in the center of the section are not connected with filaments showing that the filaments of which these cells are cross sections run parallel to the central axis. The tissues are loosely arranged, but towards the periphery dichotomous branching can be observed and these branches held together by a gelatinous secretion form the periphery.

Holdfast. — The holdfast is strong, although quite inconspicuous. It does not appear to be disc-like, but rather to be composed of branches (*Plate XL VI.*, *fig.* 7). There is a brown cellular substance which is developed beneath the holdfast. Apparently the holdfast can be developed at any point where the horizontal branch may come in contact with the substratum or at the ends of the small branches which radiate downward from the horizontal branch.

Asexual Reproduction. — Some branches appear slightly fleshier than others. When sectioned there are found distributed around them, the tetragonidia. These are developed from the peripheral cells. In one section (*Plate XL VI.*, *fig.* 8) we may find the younger gonidangia the contents of which are not yet divided, those which have divided obliquely forming two masses, and the mature tetragonidangium containing four gonidia. As has been previously stated they do not divide perpendicularly but obliquely. *Plate XL VI.*, *fig.* 8, illustrates a portion of a longitudinal section drawn from the side of the axis cylinder to the periphery. The same structures would be seen on the other side of the axis cylinder. Long narrow paraphyses extending as far again as the tetragonidangia are found which function as a protection for the gonidia. They are developed from certain peripheral cells and are made up from fourteen to sixteen cells.

Sexual reproduction. — The cystocarps are found on the branches, sometimes singly, often two on one branch, in which respect this species differs, as has been stated, from *E. hamulosa*. Beyond the cystocarps, sterile tissue extends in the form of a projection or spine. In a cross section of the cystocarp (*Plate XLVI., fig. 5*) slender branching filaments pass in and out among the carpospores. The gelatinous sheath of these filaments is not plainly seen here, the protoplasmic contents alone being visible, and the cells of the filament have become very much distorted. The carpospores are uninucleate and vary in shape, some being spherical, some oblong and others oval. The wall of the cystocarp has the characteristic structure of the wall of the frond. Distorted branches connected at times with the cortical cells are found ramifying through the structure. The development of the cystocarp has been given previously in the paper as described by Schmitz and Hauptfleisch.

The author desires to thank Professor Conway MacMillan for suggesting the subject for study, Miss Josephine E. Tilden for a detailed outline of the work, and Professor R. A. Harper for encouragement and helpful suggestions during the progress of the work.

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Explanation of Plate XLVI.

All drawings were made by the aid of a camera lucida.

1. Cross section of the frond. Central axis; small cells surrounding central axis; periphery.

2. Branching of one of the filaments taken about three cells from the central cylinder. Shows somewhat regular branching, similar to the branching of the main axis. At the periphery dichotomous branching is illustrated.

3. A portion of the central axis showing a branch which is leaving it. Protoplasmic axis of the central cylinder. The protoplasm appears to separate into threads to pass through the thickened plate.

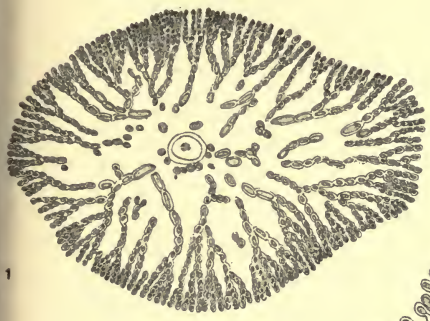
4. Longitudinal section of frond. Axis cylinder surrounded by mucilaginous sheath. This figure illustrates the point that the main branches from the axis cylinder do not run out radially, but diagonally upward, reaching the periphery opposite the lower end of the third cell of the central axis from which they started.

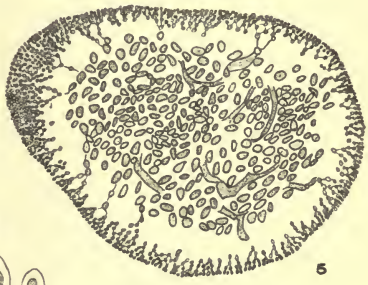
5. Cystocarp containing carpospores; filaments ramifying among he spores.

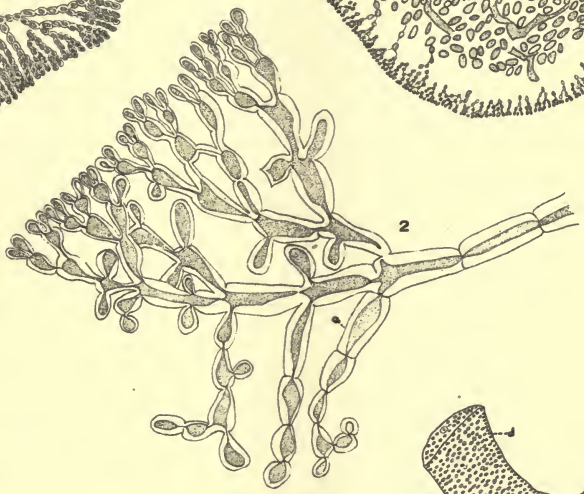
6. Carpospores and branching filaments.

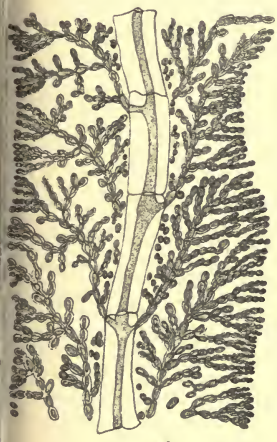
7. Holdfast. *a*, horizontal branch; *b*, stipe; *c*, brown cellular substance developed beneath the holdfast; *d*, vertical branch.

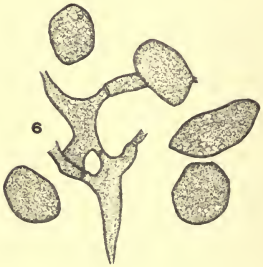
8. Section showing tetragonidangia and paraphyses in different stages.

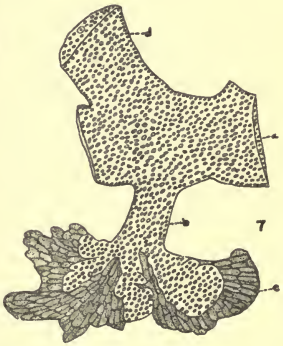


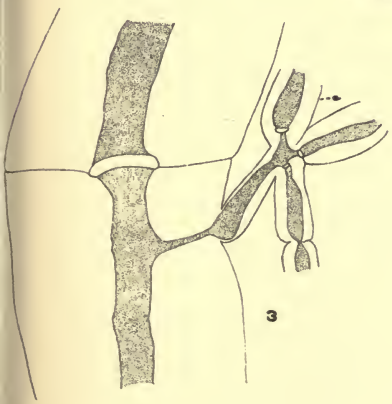












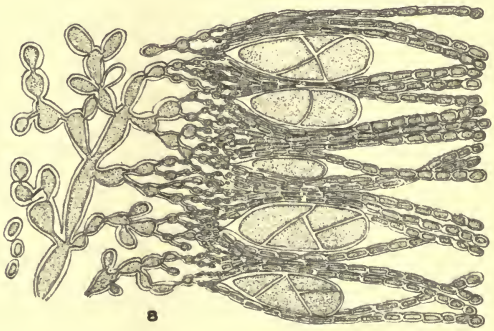


PLATE XLVI.

XXVI. OBSERVATIONS ON LAMINARIA BULLATA KJELLM.

Olga Mueller.

The plant, *Laminaria bullata*, was first studied by Kjellman in 1889 from material found by him in St. Lawrence Bay of St. Lawrence island in Behring Sea. Some of the material used for these observations was found by Miss Josephine Tilden at Tracyton, Washington, but the greater part was collected by the writer near Port Renfrew, on Vancouver island, on the straits of Juan de Fuca, in August, 1902. From this it appears that the plant has a wide distribution on the western coast of North America, extending from Alaska to Puget sound, and possibly to California.

The writer is deeply indebted to Professor Conway MacMillan and to Miss Josephine Tilden for helpful suggestions.

The plants collected at Port Renfrew were found growing attached to rocks in a narrow arm of the sea leading into a cave. Here the tidal currents were very strong, moving the plants constantly to and fro, and bringing to them the food and oxygen necessary for their life. They were found growing in the sublittoral zone and could be collected only at low tide and then with difficulty.

External Morphology.

The plant, like other members of the Laminariaceæ, consists of three portions; the holdfast, the stipe, and the lamina. The distinguishing feature of the plant is its rows of undulations, or of alternating elevations and depressions, called bullations, which run nearly parallel to the margin of the lamina, but at some distance from it. The color of the plant is dark brown. The margin of the lamina is straight and not undulated. Its texture is like that of strong, firm, sheet rubber, and its smooth glistening surface offers but little resistance to the action of the waves.

Plants of various ages were examined. The youngest was a tiny individual 2.5 cm. in length. The primitive disc showed to good advantage; its lower surface was almost flat, while the margin was slightly irregular, with indications of where the first hapteric branches would arise.

The stipe was .8 cm. long — cylindrical below and slightly flattened above where it merged into the lamina. The lamina was .17 cm. long, rather oblong in shape, showing as yet no traces of the bullations which are so characteristic of the older specimens, and the free end of the lamina even in so young a specimen was not perfect, but slightly notched and irregular — due no doubt to the action of the waves.

The largest specimen studied was found growing at Tracyton, in quiet water. Its length was 143 cm. and the greatest width of the lamina was 30 cm.

The holdfast by means of which the plant attached itself to the rocks on which it grew, consisted of a mass of dichotomously branched hapteres, which had arisen from above the primitive disc. These hapteres were brown in color, being of a lighter shade and of a more delicate texture towards the apex. In this largest specimen the stipe was unusually short, being but 2.5 cm. in length. Most of the specimens examined showed larger stipes, as in one whose lamina was 52 cm. long, the stipe had a length of 8 cm.

The stipe is strong and tough in texture. It retains the characteristic of the earlier stage of being cylindrical at the base and flattened where it merges into the lamina.

The laminæ of different specimens varied considerably in outline, some being almost oblong, others broad ovate and others elliptical.

The texture varies also, the laminæ of some plants being much thicker and firmer than of others. These differences of form and texture are due, no doubt, to differences in the intensity of the light, and the strength of the tidal currents.

The margins are in every case straight but the apex is almost always frayed and split. There is often one long split extending nearly to the base of the lamina, a second one not so deep, and several minor indentations besides. This splitting takes place in a direction parallel to the margin of the lamina and usually near the rows of bullations.

Although splitting seems to be the rule, yet our largest specimen showed scarcely a trace of it. This was no doubt due to the fact that it was found growing in quiet water. Its apex however was frayed somewhat by the action of the waves.

The region of growth is at the point where the stipe joins the lamina, and here, in the younger portion of the lamina, the bul-

lations are most perfect, being more greatly elevated and depressed, though not so large in diameter as in portions near the apex.

The plant is a perennial, and towards the older portion of the lamina the bullations become broader and more shallow until they finally disappear, leaving the older portion of the lamina with an even surface (*fig. 1*).

The greater part of the material was preserved in a five per cent. solution of formaline, a little alcoholic material was used. The former proved the more satisfactory. Free hand sections mounted in glycerine jelly were used for study.

Anatomy.

Laminaria bullata, like the other members of the Laminariaceae consists of three tissues, viz., the epidermal, the cortical, and the pith. Only the first two are found in the hapteres, while the stipe and lamina contain them all.

The surface of the plant is covered by a thick structureless cuticle; below this are the epidermal cells, prismatic in form, about one and one-half times as long as broad, but with the two shorter diameters equal, so that when the cells are seen from the surface, they appear as cubes or pentagons.

These epidermal cells are densely crowded with chromatophores, their chlorophyll being masked by the brown coloring matter characteristic of the kelps. The outer wall is comparatively thick while the lateral and inner walls are rather thin.

Below the single layer of epidermal cells are found from two to four layers of cells which are shorter and broader than the epidermal cells, and not so densely crowded with chloroplasts. These are the hypodermal cells.

Next to the hypodermal cells are found the cortical cells, which are more irregular in form, though still prismatic. They increase in size towards the center of the plant, and are followed by strengthening cells which are smaller in diameter and longer than the cortical cells. They also have thicker walls and some of them are imbedded in the mucilaginous material of the central part of the plant. These cells are devoid of chromoplasts, but contain granular protoplasm.

The pith web consists of numerous colorless, interlacing and anastomosing hyphæ embedded in mucilage.

Haptere.

A longitudinal section of the haptere shows that it is composed of a layer of cuticle on its surface and below this are the prismatic epidermal cells with their numerous chromatophores. Next to these are two layers of hypodermal cells, and just within are the cells of the cortex. These are much larger and more irregular in form than the epidermal cells (*fig. 3*). In the center of the haptere are found rather thick walled elongated cells arranged in rows.

These rows run in a straight course to the apex of the haptere (*fig. 4*), while cells at the sides of the haptere bend in curving rows from the circumference towards the center (*figs. 5, 2.*) The cells near the end of each row have the power of dividing and it is here that the haptere increases in length and in thickness. Both cross and longitudinal sections reveal numerous circular openings in the hypodermis. These are the mucilage ducts which in the haptere seems to take the form of spherical pits. Faint traces of branches may sometimes be seen, so that it is possible that these pits are in communication with each other. Each pit is surrounded by little granular secreting cells.

There is no pith in the haptere, the thick-walled, elongated cells of the cortex occupying the interior of the haptere.

In the stipe is found much the same arrangement of tissues as in the haptere, but with this difference, that a pith web occupies a considerable portion of the interior of the stipe.

The lower cylindrical portion of the stipe is hollow with only traces of the pith web left. In the upper, younger, more flattened portion, the pith web fills the center of the stipe (*fig. 6, A, B*).

The cuticle is thicker on the surface of the stipe than on the haptere; the epidermal cells are slightly more elongated but otherwise much like those of the haptere (*fig. 9*). The hypodermal cells consist of several rows and among them are found the mucilage ducts. A cross section shows them to be large elliptical openings, very close together, in fact with only the bounding cells of each duct between. Each duct appears to have been formed as a fissure between four or five adjoining cells (*fig. 8*). These cells have very granular contents, are enlarged at the ends of the ducts but compressed where the ducts are broadest. The longitudinal section shows the ducts

as long cylindrical tubes — with the inner side crenate and the outer straight, and with the secreting cells very granular (*fig.* 7).

The cortex cells of the stipe are not so large as those of the haptere (*figs.* 10, 11). The strengthening cells found near the pith web, are in general cylindrical in form; the innermost are imbedded in mucilage. These cells are characterized by each possessing an unusually large nucleus. The granular protoplasm passes through the center of the cell and communicates with that of adjoining cells through the end partition walls.

The pith web is formed of interlacing, colorless hyphæ. In both cross and longitudinal sections can be seen some hyphæ which have been cut obliquely and others which have not been cut at all, showing that they run in various directions. The majority of them take a lengthwise course, however. The hyphæ (*figs.* 12, 13) have comparatively thick walls, are imbedded in mucilage and contain protoplasm and some starch grains. Trumpet hyphæ are numerous and are found running lengthwise more often than crosswise.

In the lamina we see again the same tissues as in the stipe. The chromoplasts of epidermal and hypodermal cells are more numerous than in the corresponding cells of the stipe. The mucilage ducts are not so numerous nor so compressed, being circular rather than elliptical in outline (*fig.* 14). The cells of the cortex are large and cuboidal in form (*fig.* 14) and following these are the thick-walled strengthening cells, circular or oval in cross section (*fig.* 15), and often arranged in pairs as though recently divided; elongated and of uniform diameter in longitudinal section (*fig.* 16). In these cells the hyphæ of the pith web take their origin, as branches from the sides of the cells, or as a prolongation of the cell proper (*figs.* 15, 16).

In some cases the hyphæ can be traced from their origin in one cell, across the pith web to their termination in another cell on the other side. The pith web is richly supplied with mucilage, and imbedded in this are numerous trumpet hyphæ — which do not differ from those of the stipe (*fig.* 17).

The bullations, which are so striking a feature of the plant, are not due to a thickening of any of the tissues of the lamina, but rather to a bending in and out of these tissues, the epidermis, cortex and pith web following each other in the same order and proportion as they do in the even portions of the lamina.

Material in the fruiting condition was not available, and therefore observations on the reproduction will be made at a future time. According to Kjellman the sori are found as oval areas spread over that portion of the lamina where the old lamina merges into the new. The sporangia do not differ from those of other Laminariaceæ.

Bibliography.

Kjellman, F. J. Beringhafvets Algflora, page 46, 1889.

Explanation of Plate XLVII.

1. Photograph of *Laminaria bullata.*

2. Diagram of haptere showing the direction taken by the cells of the cortex. They run in straight lines towards the apex of the haptere, but bend outward at the sides.

3. Longitudinal section of haptere showing epidermal cells, hypodermal cells, cortex and a mucilage duct.

4. Cells near apex of haptere, as seen in longitudinal section.

5. Cells from side of haptere showing curving direction of cortex cells. Longitudinal section.

6, *a*. Diagram of lower hollow portion of stipe.

b. Diagram of upper portion of stipe.

7. Diagram of longitudinal section of stipe. *a*, epidermal region; *b*, mucilage duct; *c*, cortex; *d*, strengthening cells; *e*, pith web.

8. Cross section stipe, showing cuticle, epidermal and hypodermal cells, mucilage ducts and cortex cells.

9. Epidermal and hypodermal cells of stipe. Longitudinal section.

10. Strengthening cells of cortex of stipe. Cross section.

11. Strengthening cells of cortex of stipe. Longitudinal section.

12. Pith web of stipe. Longitudinal section.

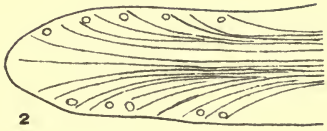
13. Pith web of stipe. Cross section.

14. Cross section of lamina showing cuticle, epidermal cells, hypodermal cells, mucilage duct and cortex cells.

15. Cross section of lamina showing strengthening cells and origin of hyphæ of pith web.

16. Longitudinal section of lamina showing same points as 15.

17. Pith web of lamina in longitudinal section.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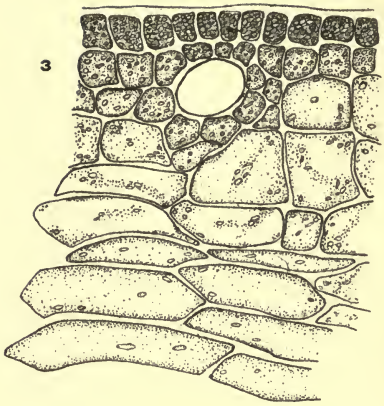
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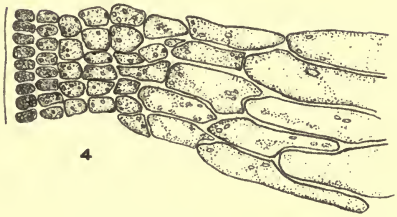
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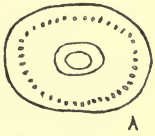
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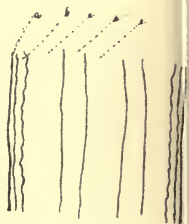

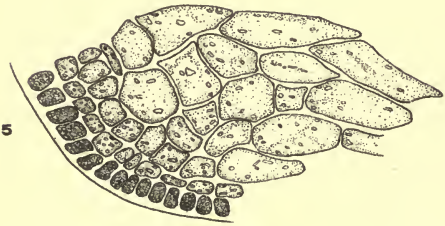
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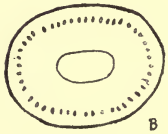
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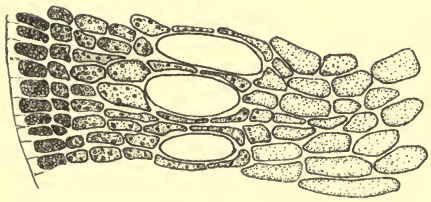

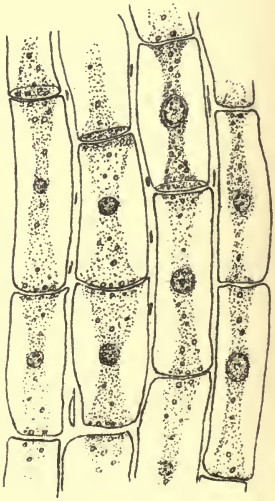
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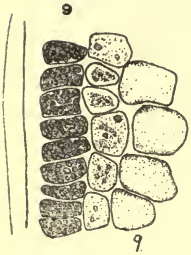
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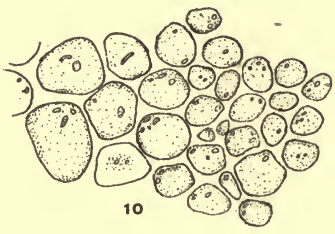
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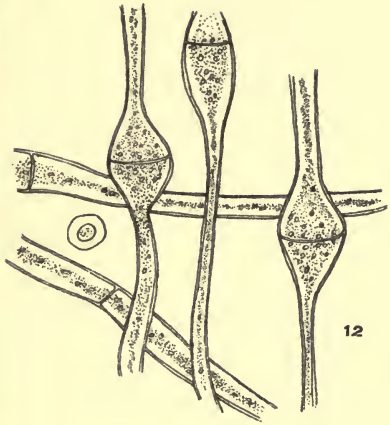

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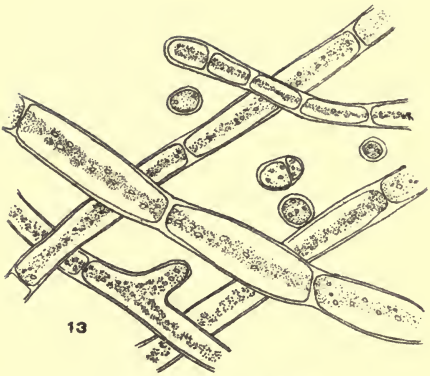

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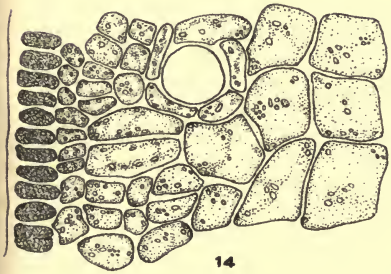

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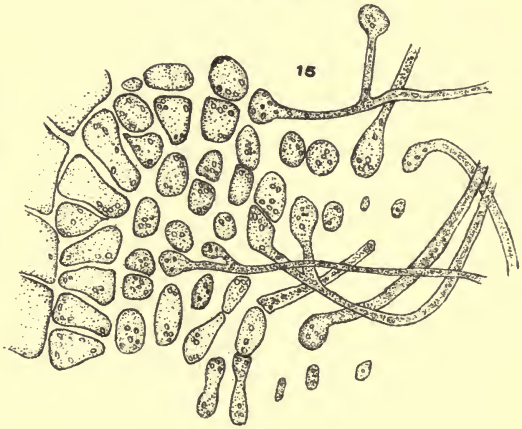

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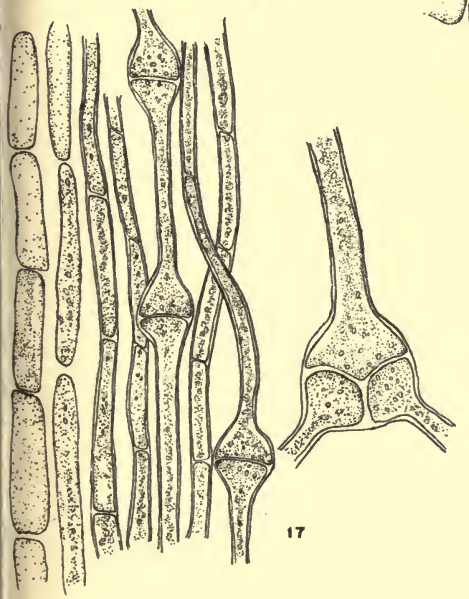

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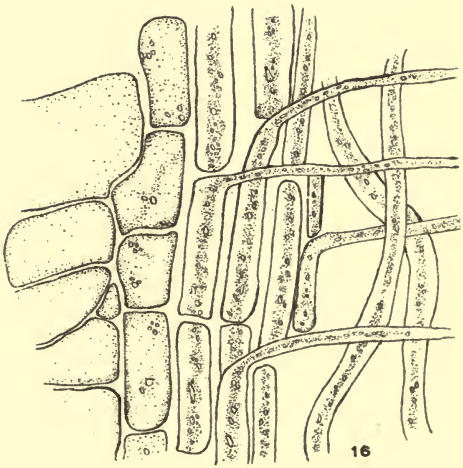

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XXVII. MINNESOTA HELVELLINEÆ.

DAISY S. HONE.

The following is a list of Minnesota Helvellineæ collected at various times since 1886.

In the determination of these species, Dr. H. Rehm's work has been carefully consulted, as have also among many others the British works of Phillips and Massee and the American papers of Peck and Morgan. These works have been used freely in the descriptions, though only where they agree with my observations on the Minnesota plants. Krombholz's plates have been compared and cited under each species. Comparisons have also been made with the Exsiccatæ in the University Herbarium and the results of such comparisons have been noted in each case. Schroeter's generic classification as outlined in Engler and Prantl, Die Naturlichen Pflanzenfamilien, has been closely followed.

All drawings were made with the aid of an Abbe camera lucida, usually from material preserved in formalin or from dried material soaked in water. In a few cases fresh material was used. The photographs were taken by Mr. C. J. Hibbard, photographer on the Minnesota Geological and Natural History Survey. All species, described are now in the collection of the Museum and Herbarium of the University. They are preserved in a mixture of two per cent. formaline and seventy per cent. alcohol and also in the dried condition.

Eight genera with fourteen species and two varieties here reported are from Minnesota. Five species belong to the Geoglossaceæ and nine to the Helvellaceæ. No member of the Rhizinaceæ has yet been reported, but the common species of *Rhizinia* probably occurs in the State. Specimens marked * are those from which the photographs have been taken. The reports of localities are given in counties.

I wish to express by thanks for the aid and supervision of Professor E. M. Freeman, of the University of Minnesota, under whose direction the work was done. I also wish to

thank Mr. C. J. Hibbard who kindly assisted by making the photographs.

1. **Helvella lacunosa** AFZEL, Act. Holm. 304. 1783. (*Plate II., figs. 11, 12, 13; Plate IV., figs. 11–16.*)

Solitary or gregarious; stipe lacunose, fistulose, slender, grayish to mouse-colored, up to 3.25 in. high, 1.25 in. wide; pileus saddle-shaped or three lobed, slightly wrinkled, mouse-colored, up to 2 in. in diameter; spores elliptical, obtuse, smooth, containing one large oil drop, 16–18 mic. long, 9–12 mic. wide; paraphyses filiform, branched, clavate, about 6 mic. wide at the tip.

On ground in moist, soft woods.

Ramsey, Sept. 1898, Freeman 229; Cass, Sept. 1898, Freeman, 186; *Hennepin, Oct. 1900, Butters 63; Hennepin, Sept. 1900, Freeman 760; Hennepin, Oct. 1900, Hibbard.

According to Phillips: "Differs from *H. crispa*, for a variety of which it may easily be taken, by the more regular pileus, 2–4-lobed, scarcely laciniate, lobes later becoming free, and especially by the colour. The stature generally smaller. Acute characters are wanting in nature, therefore it is constant." The stipe is more slender than that of *H. crispa*.

The specimens agree with Thuemen Mycoth. Univ. 809, spores of which are up to 16 × 12 mic.; Sydow Mycoth. March, 182, spores of which are 16–18 mic. × 8–10 mic.; Sydow Mycoth. March 2845, spores are 14–16 mic. × 8–10 mic.

Krombholz Schwämme III., *pl. 19, figs. 18–21* (*lacunosa*), *figs. 22–26* (*sulcata*); *pl. 21, figs. 22–24.* 1834.

2. **Helvella crispa** (SCOP.) FR. Syst. Myc. 2: 14. 1822. (*Plate II., fig. 10.*)

Generally solitary; stipe lacunated, fistulose, slightly bulbous at the base, stout, grayish, measures up to 4 in. high and 2 in. wide; pileus very much lobed and folded or wrinkled, white, up to 2.5 in. across; spores elliptical, obtuse, smooth, containing one very large oil drop, 10–16 mic. long by 8–10 mic. wide; paraphyses filiform, clavate, septate, branched, about 4 mic. wide at the tip.

On ground in hard woods, chiefly oak.

Hennepin, Oct. and Sept. 1900, Freeman 895, 734, 804; Becker, Aug. 1901, Freeman 1053; * Chisago, Sept. 1900, Butters 15; Ramsey, Sept. 1903, Cuzner; Ramsey, Sept. 1903, Wilcockson.

H. crispa is closely related to *H. lacunosa;* for differences see *H. lacunosa.*

The specimens agree with Sydow Mycoth. March 181, spores of which measure up to 12 mic. × 16 mic.

Krombholz Schwämme III., *pl. 19, fig. 27–29.* 1834.

3. **Helvella elastica** BULL. Champ. franc. 299, *pl. 242.* 1785. (*Plate II., figs. 14, 15.*)

Stipe cylindrical, not lacunated, hollow, smooth, or pruinose, grayish, up to 2.25 in. high by 1 in. across; pileus saddle-shaped, bilobed, mouse-colored above, grayish beneath, up to 1.25 in. across; spores elliptical, obtuse, smooth, containing one large, central oil drop, up to 16–20 mic. long by 10–12 mic. wide; paraphyses filiform, septate, branched, clavate, about 6 mic. wide at the tip.

On ground in moist places and among grass.

* Hennepin, Sept. 1900, Freeman 877; Hennepin, Oct. 1900, Butters 66; Becker, Aug. 1901, Freeman 1040; Cook, Aug. 1903, Freeman and Ballard 3.

The specimens agree with *D. Saccardo* Myco. Ital., spores of which average 14 mic. × 9 mic.

Krombholz Schwämme III., *pl. 21, fig. 21,* 1834.

4. **Helvella infula** SCHÄFFER, Icon. Fungi, *pl. 159.* 1763. (*Plate II., figs. 1, 2, 3; Plate IV., figs. 24–29.*)

Gregarious or solitary; small or large; stipe cylindrical, tapering toward top, at first solid later hollow, cream to flesh-colored, densely pubescent at the base, up to 2.25 in. high by 1 in. wide; pileus more or less saddle-shaped or irregularly undulated, margin attached in places to the stipe, yellow to cinnamon brown or chestnut brown above, cream to flesh-colored beneath, finely pubescent beneath, up to 2.25 in. deep; spores elliptical, obtuse, smooth, with two equal oil drops, 18–24 mic. by 8–12 mic.; paraphyses clavate, septate, branched, brownish, about 4 mic. wide.

On the ground along paths and trails or among moss. It usually prefers clayey ground but occasionally is found even on decayed logs.

* Cook, Aug., 1902, Fink; Cook, Aug., 1903, Freeman and Ballard 5.

This is *Gyromitra infula* (Schäff.) Buel. Schröter in Die Naturlichen Pflanzenfamilien, has defined *Gyromitra* as possessing an inflated cap. In the above species no true inflation of the cap is found, *i. e.*, the cavity of the stipe is not prolonged into a cavity in the cap as in the morels. Hence the condition here is similar to that of *Helvella.* It approaches the true *Gyromitras* however in the tendency of the cap to become irregularly convoluted and especially in the fusion of the cap with the stipe. *Helvella infula* Schäff. therefore stands between the *Gyromitras* with truly inflated cap and the typical *Helvellas* without fusion of cap and stipe. There is moreover often a very marked saddle form to specimens of this species. Schröter's conception of the genus *Gyromitra* accords with Fries' original description "discus bullato-inflatus, costis elavatis gyrosus." (Summa Veg. Scand. 346. 1849.)

Our specimens agree with Krombholz Schwämme III., *pl. 19, figs. 11–13* (*H. rhodopoda* which is clearly a true *Helvella* and is included by Rehm as a synonym under *H. infula.* They also agree with Roumeguere Fungi Gall. Exsicc. 1208, in which the spores measure 18–22 mic. × 9–12 mic.; Krombholz Schwämme III., *pl. 21, figs. 12–17. Helvella infula* also agrees as far as exterior views are concerned but the sectional views do not clearly show the relation of stipe and cap cavities as seen in the Minnesota specimens.

Certain specimens of these *Helvellas* contained an abundance of *Sphæronamæmella helvellæ* Karst. It differs from Karsten's descriptions (Hedw. **23**; 18. 1884) in its possession of ultimately uniseptate spores. These are at first continuous and later uniseptate. In all other points the agreement is complete.

5. **Verpa conica** (Mill.) Swartz. Vet. Ak. Handl. 136. 1815. (*Plate II., figs. 4, 5, 6; Plate IV., figs. 30–33.*)

Stipe cylindrical, tapering at the apex, hollow, white, even or slightly wrinkled, floccose, up to 2.5 in. high and 1.5 in. wide; pileus conical, even or slightly wrinkled, brownish above, white beneath, up to .5 in. deep by 1 in. across at the base; spores elliptical, obtuse, smooth, containing one large central oil drop, 20–22 mic. long by 10–14 mic. wide; paraphyses filiform, septate, branched, 6 mic. wide.

On moist ground.

Ramsey, May 1899, Freeman 313; * Wright, May 1900, Freeman 588; Beltrami, May 1902, L. R. B. W. 52; Ramsey, May 1903, Polley; Hennepin, May 1903, Ramsey and Hone; Chisago, May 1903, Nelson.

The specimens agree with Sydow Mycoth. March 3166, of which the average spore measures 20 mic. × 12 mic.; Roumeguere Fungi Selecti Exsicc. No. 4554, of which the average spore measures 20–22 mic. × 12–14 mic.

Krombholz Schwämme III., *pl. 5, figs. 29–31.* 1834.

6. **Verpa bohemica** (KROMBH.) SCHRÖT. Schles. Krypt 3^2: 25. 1893. (*Plate II., figs. 7, 8, 9; Plate IV., figs. 17–23.*)

Stipe cylindrical, stuffed or hollow, floccose, white, even, not wrinkled, up to 4 in. high by 1 in. across; pileus conical obtuse, reticulated or longitudinally ribbed, edge sometimes inflexed so as to form a white border, brownish above and white beneath, up to 1.25 in. deep; spores elliptical, obtuse, straight, or bent, one-celled, hyaline, only two in an ascus, up to 60 mic. long by 16 mic. wide; paraphyses filiform, branched, septate, up to 4 mic. wide at the tip.

On damp ground, often among broken limestone.

* Hennepin, May 1899, Freeman 302; Hennepin, April 1901, Lyon; Hennepin, April 1903, Butters and Ramsey; Ramsey, May 1903, Polley.

Verpa bohemica is easily distinguished by the two large spores in each ascus.

The specimens agree with Rathay Fl. Exsicc. Austro-Hung. No. 1573, of which the spores measure 60 × 14 mic.; Thuemen Mycoth. Univ. 609, of which the spores measure 60–80 mic. × 16–18 mic.

Krombholz Schwämme III., *pl. 15, figs. 1–13.* 1834.

7. **Morchella hybrida** (SON.) PERS. Syn. Meth. Fungi. 620. 1801. *Plate I., figs. 7, 8; Plate IV., figs. 8–11.*

Stipe cylindrical, bulbous at base, tapering toward apex, whitish, granulose, 1–6 in. high: pileus brownish to tan, conical, acute, lower half free from stipe, longitudinally pitted and ribbed, about 1.5 in. deep; spores elliptical, obtuse, smooth, hyaline, 18–20 mic. long by 10–14 mic. wide; paraphyses slightly clavate, vacuolated, septate, branched, about 12 mic. wide at the tip.

On ground in shady places, sometimes in gravel and also along roadsides.

* Hennepin, May 1902, Butters 201; * Hennepin, May 1903, Polley; * Ramsey, May 1903, Freeman; Hennepin, May 1903, Lyon and Rosendahl.

Morgan states (Morchellæ—The Morels, Journ. Myc., Vol. VIII., June, 1902) that no paraphyses are present in *M. hybrida* or in *M. esculenta*. *M. hybrida* as generally accepted however has paraphyses. They are large and are therefore easily mistaken for young asci, but are septate and sometimes branched. Collections marked with a * contain small forms, one inch high or less.

The specimens agree with Thuemen Mycoth. Univ. No. 412, the spores of which measure 20–24 mic. × 14 mic.; Ellis and Everhart N. A. Fungi No. 2628, the spores of which measure 20–22 mic. × 12–14 mic.

Krombholz Schwämme III., *pl. 15, figs. 14–21.* 1834.

8. **Morchella esculenta** (L.) Pers. Syn. Fungi. 618. 1801. (*Plate I., figs. 3–9; Plate IV., figs. 1–6.*)

Solitary or gregarious; stipe cylindrical, hollow, sometimes bulbous, granulose or glabrous, white, entire, .5 to 2 in. high by .5 to 1 in. thick; pileus is very varied from conical to obtuse, irregularly or longitudinally pitted, olive brown to grayish brown; ribs of the pileus are thick and obtuse at the edge; the surface being even, pileus is about 2.5 in. long; spores are elliptical, obtuse, smooth, one large oil drop in center, sometimes yellowish, 14–22 mic. long by 8–14 mic. wide; paraphyses filiform, clavate, septate, branched, in some specimens very abundant.

On the ground in shady woods. Very common in oak woods.

* Hennepin, May 1891, Sheldon 20; Wright, May 1900, Freeman 675; Hennepin, May 1901, Polley; Hennepin, May 1903, Rosendahl; Wright, May 1903, Polley; *Hennepin, May 1903, Hone 218; * Hennepin, May 1903, Polley.

Like *M. hybrida*, *M. esculenta* is generally accepted as having paraphyses. The above cited material certainly possesses structures which resemble paraphyses in all essentials. They are septate, often branched, and are smaller than the asci, growing among the latter in the hymenium. The collections marked with a * have rather conical caps and the ribs are longitudinal and regular. In all other characteristics they are true esculenta forms and I have no doubt that these two collections contain the forms which have been described as *M. conica*. As

M. esculenta occurs in such varied sizes and forms in all of the material which I have examined, I consider that if these are *M. conica*, that they are only a form or variety of *M. esculenta* and not a distinct species. *Plate I., figs. 3–6*, show photographs of both forms.

The specimens agree with Thuemen Fungi Austr. 12, 13, where the spores measure 14–22 mic. × 6–12 mic (12 contains paraphyses measuring 10 mic. at tip and branched); Rathay Fl. Exsicc. Austro-Hung. 1572, in which the spores measure 20 × 12 mic.; D. Saccardo Mycoth, Italica, 507, in which the spores measure 14–18 × 10–12 mic.

Krombholz Schwämme III., *pl. 16, figs. 3, 4* (Morch. escul. rotunda Fr.), *5, 6* (Morch. escul. vulgaris F.), *pl. 17, figs. 3–4* (Morch. escul. fulva Fr.), *9–16; pl. 19, figs. 6–7.*

9. **Morchella crassipes** (VENT.) PERS. Syn. Meth. Fungi 620. 1801. (*Plate I., figs. 1, 2; Plate IV., fig. 7.*)

Solitary or gregarious; stipe cylindrical, hollow, bulbous, very much furrowed, granulose, white, .5–4.5 in. long by .75–1.75 in. wide; pileus conical or subconical, ribs very irregular, undulating, thick, acute at the edge, pits deep, surface very ragged and uneven, yellowish, 1.5–3 in. deep; spores elliptical. obtuse, smooth, one large central oil drop, 20–22 mic. long by 10–12 mic. wide; paraphyses broad, as wide as the asci, septate and vacuolated, 10–18 mic. wide.

On ground in most shady woods or open places.

LeSueur, June 1891, Taylor 49; Ramsey, June 1899, Freeman 359; Hennepin, May 1899, Buell; Hennepin, May 1901, Freeman 1000¾; * Hennepin, May 1903, Hone 217.

M. crassipes differs from *M. esculenta* in its greater size, up to 7.5 in. high, yellowish color and very uneven surface. The ribs also are very acute at the edge and thick at the base while in *M. esculenta* they are even and very obtuse at the edge.

Krombholz Schwämme III., *pl. 16, figs. 1–2.* 1834.

GEOGLOSSACEÆ.

10. **Spathularia clavata** (SCHAEFF.) SACC. Michelia 2: 77. 1880. (*Plate III., fig. 1; Plate V., figs. 13–15, 20.*)

Gregarious; stipe cylindrical slightly compressed, hollow, erect, single or cæspitose, fleshy, yellowish brown, up to 3 in. long by .5 in. wide; pileus spatulate or broadly clavate, gen-

erally obtuse, much compressed, running down the stipe for some distance on opposite sides, hollow, glabrous, margin undulated, surface wavy or slightly lacunose, yellowish, up to 1.25 in. wide by 1.5 in. long; spores parallel in fascicles, hyaline, linear-clavate, slightly bent, multiseptate, containing small oil drops, up to 45 mic. long by 2 mic. wide; paraphyses filiform, septate, branched, tips not thickened but wavy, up to 2 mic. wide, numerous.

On rotten wood among moss.

St. Louis, July 1886, Arthur 194; * Cook, Aug. 1903, Freeman and Ballard 20.

These specimens agree with Thuemen Fungi Austr. 925, in which the spores measure up to 50 mic. × 2 mic.; Sydow Mycoth. March 2516, in which the spores measure 45–60 mic. × 2 mic.; A. Kerner Fl. Exsicc. Austro-Hung. 1874, in which the spores measure 40–50 mic.; Ellis N. A. Fungi 1268, in which the spores measure 40–60 mic. × 2 mic.

Krombholz Schwämme III., *pl. 5, fig. 22.* 1834.

11. **Geoglossum hirsutum** Pers. Comm. Schaff. Icon. Fungi Bav. 37. 1800. (*Plate III., fig. 5; Plate V., figs. 1–4.*)

Cæspitose, erect, black: stipe cylindrical, solid, even, black, hirsute, up to 2.25 in. long by 1 in. wide; pileus club shaped, compressed or plicate, distinct from stipe, hirsute, up to 1 in. long and .75 in. wide; spores linear, slightly curved, septate into about 16 cells, multiguttulate, brown, obtuse, 100–120 mic. long by 4–7 mic. wide; paraphyses filiform, septate, curved or arched at the tip, much enlarged, brownish, 3 mic. wide, tip 4–6 mic. wide; setæ rigid, simple, brown or black, intermingled with the asci, sometimes twice as long as the asci, projecting beyond.

On moist ground among grass.

* Washington, July and Sept. 1903, Lyon; Washington, July 1903, Wheeler.

The specimens agree with Jaczewski, Komarov, Franzchel. Fungi Rossiæ Exsicc. 245, in which the spores measure 100–135 × 4 mic.; Sydow Mycoth. March 1069, in which the spores measure 100–120 × 4 mic.

They agree with the figures and description of Massee, Monograph of Geoglossaceæ, Ann. Bot., Vol. 11, *Pl. XIII., figs. 78, 79*; *Pl. XII., figs. 31, 31, a, 32.* 1897; Krombholz Schwämme III., *Pl. 5, figs. 20–21.* 1834.

Geoglossum hirsutum americanum COOKE, Mycogr. 3. *fig.* 1875.

Hymenium appears glabrous when examined under a hand lens as the setæ project but slightly beyond the asci; spores light brown and only 7–10 septate. It agrees with *G. hirsutum* in all other characters.

On ground among moss in an alder swamp.
Sherburne, Aug. 1901, Polley.

12. **Leptoglossum luteum** (PK.) SACC. Syll. Fungi 8: 48. 1889. (*Plate III., fig. 2; Plate V., figs. 16–19.*)

Stipe cylindrical, minutely scaly, whitish, stuffed, even, up to 1 in. high; pileus club shaped, slightly compressed, grooved on one side, yellowish, smooth, up to .5 in. high; spores oblong, slightly curved, hyaline, obtuse, 28–38 mic. long by 5–6 mic. wide (most of the spores were immature but some show distinct indications of septations); paraphyses filiform, clavate, curved at the tip, branched, numerous, about 2 mic. wide.

On ground in swamp.
* St. Louis, July, 1886, Arthur 195.

The specimens agree with a specimen of unknown origin No. 82 collected at Kirkville, N. Y., June, 1889, the spores of which measure 26–34 × 4–5 mic. and each cell often contains one large oil drop.

They agree with the plates and descriptions of Peck, Rep. N. Y. St. Mus. Nat. Hist. **24**: 94, pl. 3, *figs. 20–24.* 1872 and Massee Monograph of Geoglossaceæ, Ann. Bot., Vol. 11, *pl. XII., figs. 28, 29, 30.* 1897.

13. **Leotia lubrica** (SCOP.) PERS. Syn. Meth. Fungi 613. 1801. (*Plate III., fig. 4; Plate V., figs. 5–8*).

Gregarious, cæspitose, usually gelatinous especially when old; stipe cylindrical or inflated at the base, compressed and often bent, hollow or pulpy within, pruinose, yellowish to greenish, up to 2 in. long by .5 in. wide; pileus irregularly hemispherical, inflated, wavy margin obtuse and folded, yellow to yellowish olive-green and very dark green, up to 1 in. across; spores elliptical slightly acute, straight or bent, smooth, with several large oil drops, when mature 2–4-celled, colorless or slightly greenish, often in two rows, 4–5 mic. wide by 18–22 mic. long; paraphyses filiform, septate, branched, slightly clavate, hyaline or greenish, up to 2 mic. wide, 4 mic. wide at tip.

On ground in woods often among moss. Usually on sandy soil.

Hennepin, July 1903, C. C. Conser; Washington, Aug. 1903, Wheeler; Ramsey, Sept. 1903, Freeman 1380.

The collection 1380 Freeman agrees in color and form with *L. chlorocephala*. The stipe is green and only slightly lighter than the cap and the plants are more slender. As many gradations may be found between this and typical *L. lubrica* forms so all of the above specimens have been listed under *L. lubrica*. The dried material from collection 1380 Freeman shows a yellowish stipe.

The specimens agree with Thuemen Fungi Austr. 517, the average spores of which measure 20 × 5 mic.; Sydow Mycoth. March 278, 667, the average spores of which measure 20 × 4 mic.; Thumen Mycoth. Univ. 1112, the average spores of which measure 18–20 mic. × 4–5 mic.

They agree with the descriptions and figures of Rehm Krypt. Fl. Vol. I. 1161. *fig. 1–4.* 1896 and of Massee Monograph of Geoglossaceae, Ann. Bot., Vol. 11, *pl. XIII.*, *figs. 61*, *65.* 1897.

14. **Cudonia circinans** (Pers.) Fr. Summa Veg. Scand. 348. 1849. (*Plate III., fig. 3; Plate V., figs. 9–12*).

Gregarious, erect, somewhat cæspitose; stipe fistulose or solid, even, twisted, expanding upward with the pileus, granulose or powdery, darker colored than pileus, up to 2 in. long, .25 in. across; pileus fleshy, convex and undulated, margin free, involute, variable in color with age, tan to dingy yellow, sometimes flesh tinted, up to .5 in. broad; spores linear, multiseptate, often containing several small oil drops in each cell, hyaline, curved slightly when free, obtuse, 35–50 mic. long by 2 mic. wide; paraphyses filiform, branched, septate, tip not enlarged but curved, up to 2 mic. wide.

On decayed log in deep balsam fir woods.

* Cook, Aug. 1903, Freeman and Ballard 131.

The specimens agree with Thuemen Mycoth. Univ. 1809, the spores of which measure 35–45 × 2 mic.; Ellis and Everhart N.A. Fungi 3533, the spores of which measure 35–40 × 2 mic.; D. Saccardo Mycoth. Italica 873, the spores of which measure 35–45 × 2 mic.

They also agree with the descriptions and figures of Rehm Raben. Krypt. Fl. Vol. I., 1163, *fig. 1–4.* 1896 and Massee

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Explanation of Plates.

All figures of plates are natural size.

Plate XLVIII.

Figures 1, 2. *Morchella crassipes.*
Figures 3, 4. *Morchella esculenta.*
Figures 5, 6. *Morchella esculenta* form *conica.*
Figures 7, 8. *Morchella hybrida.*

Plate XLIX.

Figures 1, 2, 3. *Helvella infula.*
Figures 4, 5, 6. *Verpa conica.*
Figures 7, 8, 9. *Verpa bohemica.*
Figure 10. *Helvella crispa.*
Figures 11, 12, 13. *Helvella lacunosa.*
Figures 14, 15. *Helvella elastica.*
Figure 16. See paper by Miss Jessie Polley on *Physalacria.*

Plate L.

Figure 1. *Spathularia clavata.*
Figure 2. *Leptoglossum luteum.*
Figure 3. *Cudonia circinans.*
Figure 4. *Leotia lubrica.*
Figure 5. *Geoglossum hirsutum.*

Plate LI.

Figures 1–6. *Morchella esculenta.*
Figure 1. Longitudinal section ½ nat. size.
Figure 2. Ascus, × 195.
Figure 3. Ascus with sixteen spores, × 195.
Figure 4. Spores, × 387.
Figure 5. Apex of an open ascus, × 387.
Figure 6, *a.* Normal mature paraphyses, × 195.
Figure 6, *b.* Young paraphyses, × 195.
Figure 7. *Morchella crassipes*, longitudinal section, ½ nat. size.
Figures 8–11. *Morchella hybrida.*
Figure 8. Longitudinal section, ½ nat. size.
Figure 9. Ascus, × 195.
Figure 10. Spore, × 387.
Figure 11. Paraphyses, × 195.
Figures 12–16. *Helvella lacunosa.*
Figure 12. Cross section of stipe, ½ nat. size.
Figure 13. Paraphyses, × 195.
Figure 14. Ascus, × 195.

Figure 15. Young ascus, × 195.
Figure 16. Spores, × 387.
Figures 17–23. *Verpa bohemica.*
Figure 17. Two asci, one emptied of spores, × 195.
Figure 18. Ascus, × 195.
Figure 19. Paraphyses, × 195.
Figure 20. Young paraphyses, × 195.
Figure 21. Longitudinal section, ½ nat. size.
Figure 22. Spores, × 387.
Figure 23. Open ends of asci, × 195.
Figures 24–29. *Helvella infula.*
Figure 24. Longitudinal section, ½ nat. size.
Figures 25, 26. Paraphyses, × 195.
Figure 27. Spores, × 387.
Figures 28, 29. Asci, × 195.
Figures 30–33. *Verpa conica.*
Figure 30. Longitudinal section, ½ nat. size.
Figure 31. Ascus, × 195.
Figure 32. Spores, × 387.
Figure 33. Paraphyses, × 195.

PLATE LII.

Figures 1–4. *Geoglossum hirsutum.*
Figure 1. Spore, × 387.
Figure 2. Ascus, × 195.
Figure 3. Hair, × 387.
Figure 4. Paraphyses, × 195.
Figures 5–8. *Leotia lubrica.*
Figure 5. Longitudinal section, ½ nat. size.
Figure 6. Ascus, × 387.
Figure 7. Spores, × 387.
Figure 8. Paraphyses, × 387.
Figures 9–12. *Cudonia circinans.*
Figure 9. Longitudinal section, ½ nat. size.
Figure 10. Ascus, × 387.
Figure 11. Spores, × 387.
Figure 12. Paraphyses, × 387.
Figures 13–15, 20. *Spathularia clavata.*
Figure 13. Longitudinal section, ½ nat. size.
Figure 14. Ascus, × 387.
Figure 15. Spores, × 387.

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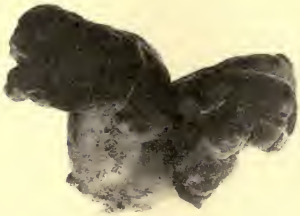

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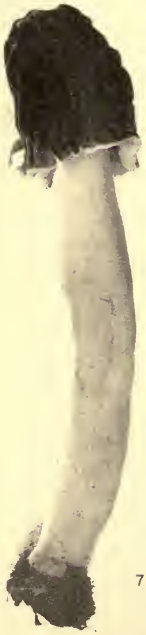

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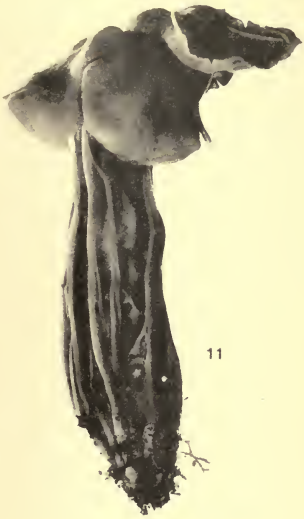

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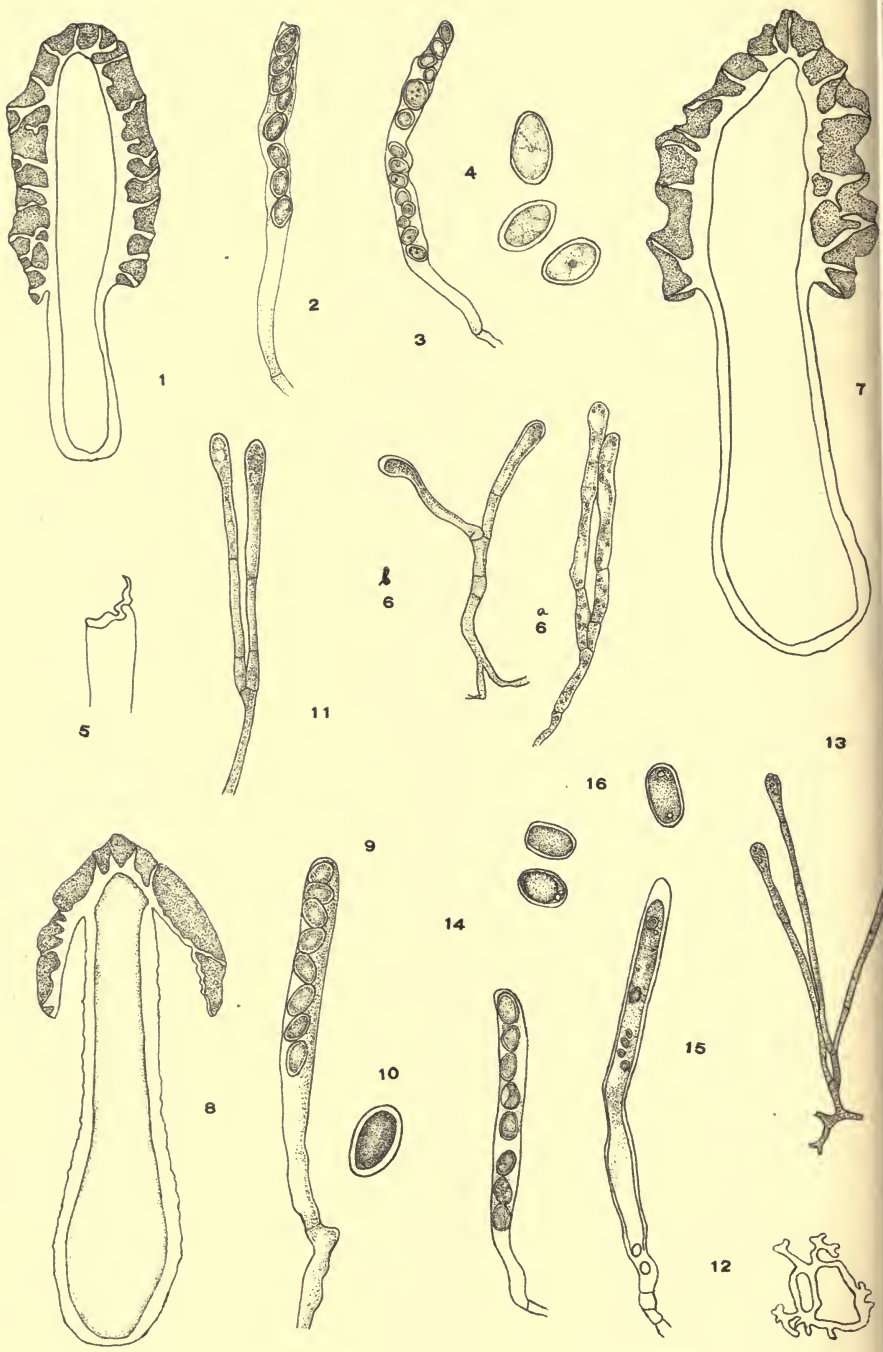
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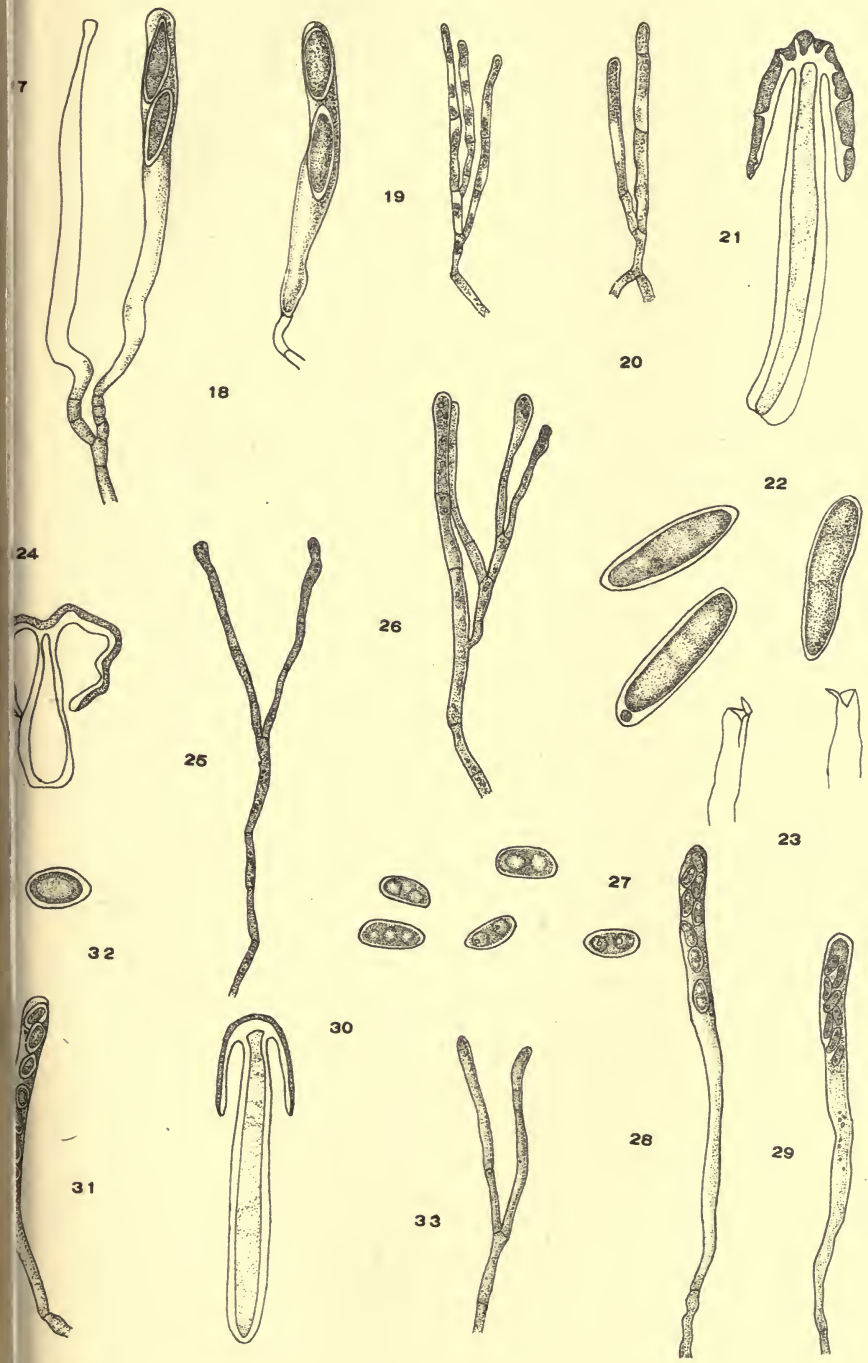
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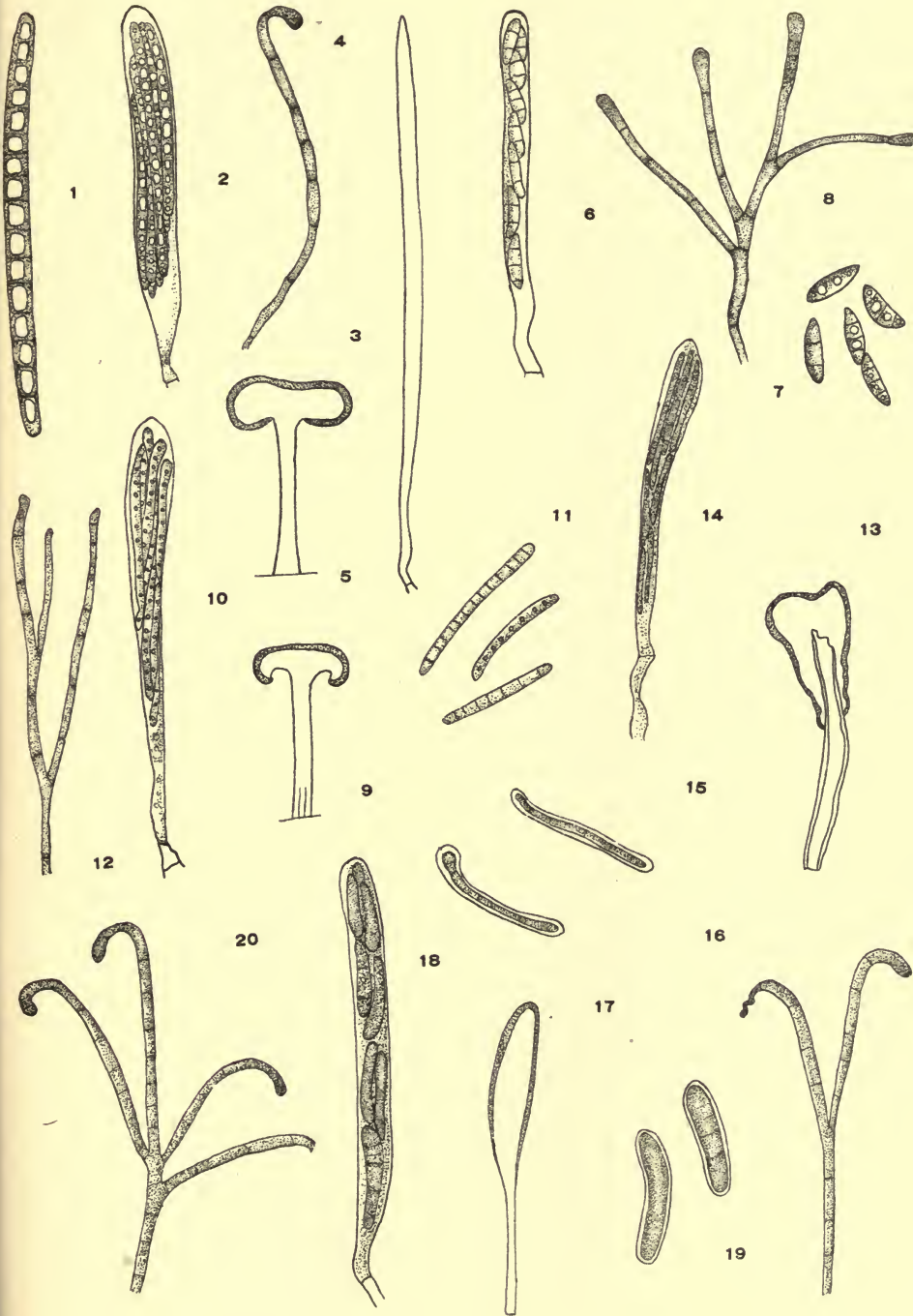
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XXVIII. OBSERVATIONS ON PHYSALACRIA INFLATA (S.) PECK.

Jessie M. Polley.

This rather rare and interesting club fungus was first described by Schweinitz in 1822, under the name of *Leotia inflata* (1). In 1828 Fries described it as *Mitrula inflata* (2), and it was again described by Cooke in 1879 as *Spathularia inflata* (3).

The first accurate description however, and the first appreciation of the true position of this fungus, was given by Peck (11) in 1882. His description is as follows:

"**Physalacria** gen. nov.

("From φυσαχις, a bladder and ακρα, the top.)

"Club subglobose, inflated, thin, somewhat tenacious, everywhere covered by the hymenium, supported on a distinct, slender stem.

"Distinguished from *Pistillaria* by the thin, inflated bladder-like club and the distinct slender stem. The following is at present the only species known:

"Physalacria inflata.

"White, becoming tinged with yellow; club subglobose, submembranaceous, glabrous, flaccid, more or less uneven with irregular depressions or wrinkles, two to four lines broad; stem slender, equal, firm, straight, solid, four to nine lines high, minutely hairy or subfurfuraceous, mostly cespitose; spores minute, narrowly elliptical, colorless, .00016–.0002 of an inch long and about half as broad.

"Decaying wood and bark in woods and shaded places. It occurs especially in mountains or hilly districts in summer."

Two years later Farlow (8) collected a few specimens in the White Mountains and identified them with Peck's description. His account is as follows:

"PHYSALACRIA INFLATA (S.) Peck.

"On logs in wet places. Shelburne, a small number of specimens of this curious species were found on a log in a brook which was nearly dry. My specimens were in fruit and I am able to confirm the account of the fructification given by Peck in Bull. Torr. Bot. Club, Jan. 1882. The species does not belong to the genus *Mitrula* where it was placed by Schweinitz, but it is one of the Hymenomycetes, closely related to *Pistillaria*, as correctly shown by Peck. I could find only two spores to a basidium."

The material from which the present description is written was collected by E. M. Freeman at Detroit, Minn., in August, 1901. A part of the material used had been preserved in 2½ per cent. formaline and the rest had been dried. Permanent slides were prepared by the ordinary paraffin section method and gentian violet and bismark brown were employed in staining. The sections from which the greater part of the work was done were cut with an ether freezing microtome and mounted in water or in glycerine. All detail drawings were made with the aid of an Abbe camera lucida.

The plant grows upon decaying wood in shaded places or in deep woods, though it seems not to need as much moisture as many of the club-fungi. It grows in clusters and is ⅜ to ⅝ in. in height. Stem $\frac{1}{20}$ and club ½ inch thick (Pl. XLIX., fig. 16). Many stipes come up together and diverge toward the top. The plants spread apart by the enlargement of the clubs which fuse on the sides where they press against each other. In general appearance they simulate some of the ascomycetes of the Geoglossacæ, *e. g.*, *Spathularia* and *Mitrula*, which led to the assignment of this species under those genera by earlier authors. They are creamy white in color except the base of the stem which is black.

Peck's description is very apt. In addition to the characters pointed out by him, the following are worthy of note. The stem is tough, at least in formaline material, and the central portion is more stringy than the peripheral region, this central portion is composed of two distinct kinds of hyphæ, small even threads with septa far apart (*figs. 7, 12*) and larger hyphæ which are composed of flasked-shaped cells set end to end. Both kinds of hyphæ are very rarely branched and lie parallel. In

cross section all of the cells appear circular and the largest are 11 μ in diameter and four or five times as large as the smallest ones. The latter are 2.5 to 4 μ in diameter and are probably stereome in function, while the former have perhaps to do mainly with conduction. There are, even in the young stems, fairly large air spaces between the hyphæ. The outer portion of the stipe consists of a looser weft of septate branching hyphæ which produce large flask-shaped cells, (7–9 μ × 22–40 μ) pointing outward and giving to the surface a hairy appearance. The stipe is hollow when old.

The club consists of a very thin wall enclosing a gelatinous interior (*figs. 2, 3*). The subhymenial layer is composed of very fine, septate, closely-woven, much branched hyphæ. On the inside of this layer the hyphæ float out in single branching strands which show abundant clamp-connections (*fig. 1*). The clamp-connections appear to agree with those described by Brefeld (9) in *Coprinus*, rather than with those which Harper (10) observed in *Hypochnus*. For in the latter stages walls are formed at both ends of the clamp cell rather than only at the end from which the clamp-cell originated. In some cases the branch which forms the clamp is of considerable length, forming a large loop (*fig. 5*). It was also observed that in many cases the ends of approaching hyphæ are fused, forming a similar figure to that of a large clamp (*fig. 9, a*). The hymenial layer shows differentiation into three kinds of cells. The "paraphyses" which are probably immature basidia, are small and closely packed (*fig. 4*). Large flask-shaped cells prolonged into blunt spine-like processes project outward. Some of them do not reach the surface of the cap while others project beyond (*figs. 1, 10*). They are of the same shape as the hair-like cells of the stem and seem to serve the usual protective purpose of the cystidia of the lower hymenomycetes. They are probably also special excretory cells as is indicated in the abundance of calcium oxalate crystals excreted and in the sunken position of many. The outer ends of these cells are covered by an irregular cap of crystals (*figs. 6, 8*). A third distinct type of cells in the hymenium are the basidia. They are somewhat thicker than the "paraphyses" and the sterigmata protrude beyond the surface. Peck and also Farlow described for this species two-spored basidia. This material shows, however, that the latter carry from two to four spores. Numerous

examples were noted of basidia bearing either three or four sterigmata. The basidia are barrel-shaped and considerably larger in diameter than the hyphæ from which they arise. The sterigmata are borne at the summit in the usual manner for hymenomycetes. The spores are oval in shape and about 1.6 × 2.5 μ in size.

Physalacria inflata apparently enjoys a wide distribution but seems never to occur in great abundance. It has been collected in S. Car. (1), the White Mountains (8), New York (11), and in Minnesota. As far as I know these are the only recorded occurrences.

Systematic Position.

Physalacria has been assigned to the Clavariaceæ. Among the genera of this family *Baumanviella* (P. Hennings) seems to be most nearly related, though this genus contains single-spored basidia. *Glœocephala* (Massee) differs considerably from *Physalacria*, not only in one-spored basidia but also in the localization of the hymenium on the lower surface of the more or less flattened club and in the prominence of its cystidia-like hairs on the upper surface. *Pistillaria* and *Typhula*, on the other hand, approach in form and texture the true *Clavaria* types. In certain aspects *Physalacria* also shows interesting resemblances to the Trembling fungi, *e. g.*, to such forms as *Ditiola* amongst the Dacromycetineæ. In *Physalacria* a somewhat gelatinous region is found below the subhymenium, though this gelatinous region is not extensively developed. The forked basidium of *Ditiola* removes it far from *Physalacria*, yet the partial gelatinization of the club of the latter is one of but a few such instances among the *Clavariaceæ*.

Other Species.

Physalacria langloisii E. & E. (4) is a very small species found in Alabama in 1888. It grows on rotten wood and is less than 1 mm. in height, and is distinguished from *P. inflata* (S) Peck, by the smaller size and urn-shaped cystidia.

P. orinocencis Pat. (5) is also a small species about 3 to 4 mm. high. Found in northern South America. It grows in clusters, has an inflated club which appears (from figures in Nat. Pflanzenfamilian Fungi, 1 [1] **: 131. 1898) to be more or less conical in form and its hymenium covers the whole surface of the club.

P. stillboidea (Cke.) Sacc. (7) was described by Cooke (6) (under *Pistillina stillboidea* Cooke) from New Zealand material on *Panax* leaves. It is also very small, about 3 mm. in height, with hollow, globose, depressed club. Cooke (6) also states that *Pistillina paradoxa* B. & C. (*Crinula paradoxa* B. & C.) must also be referred to the same genus as *Pistillina stilboidea* Cooke. Among others he cites as exsiccatæ Thümen Myc. Univ. No. 208, and Ellis N. A. Fungi No. 23 on living leaves of *Quercus*. These two specimens have been examined and prove to be *Cronartium asclepiadeum quercinium* B. & C. They agree with Ellis and Everhardt N. A. Fungi No. 1881, second series.

The genus *Physalacria* as at present known therefore, comprises four species, two North American, one South American, and one Australian.

Figure on Plate XLIX.

Figure 16. Photograph of plant, natural size.

Description of Plate LIII.

Figures 2 and 3 are magnified 5.5 all the remaining figures are magnified 455 times.

Figure 1. Detail of section vertical to surface of club.

Figure 2. Diagram of vertical median section through whole plant; *a*, hymenium; *b*, closely packed hyphæ; *c*, interior gelatinous portion; *d*, layer of flask-shaped cells; *e*, interior portion shown in fig. 7; *f*, hollow central portion of old stem.

Figure 3. Same as fig. 2 except that it is taken at right angles to flat surface. Lettering same as fig. 2.

Figure 4. Small portion of hymenium showing basidia with spores and also sterile cells between.

Figure 5. Clamp connections of loose inner hyphae. Stages in formation.

Figure 6. Detail of flask-shaped cells on surface of stipe.

Figure 7. Cross section of inner portion of stipe. *a*, intercellular space; *b*, large cells; *c*, smaller cells.

Figure 8. Cystidial cell of hymenium.

Figure 9. Peculiar hyphal septations; *a*, anastomosing of two approaching hyphal ends.

Figure 10. Cross section of outer portion stipe; *a*, flask-shaped cells; *b*, hyphæ beneath the surface.

Figure 11. Longitudinal section of portion shown in fig. 10.

Figure 12. Detail of large cells in stipe.

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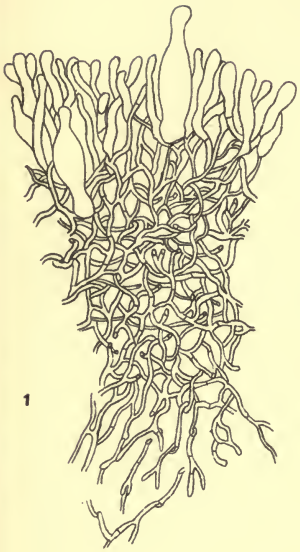

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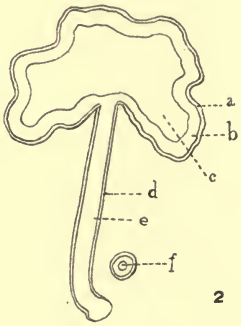

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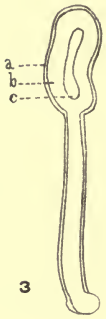

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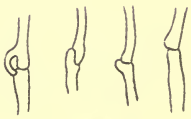

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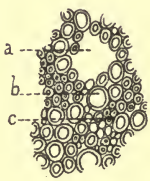

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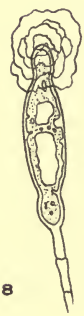

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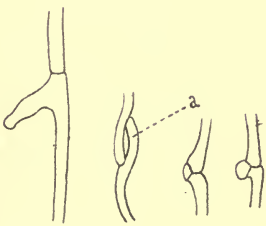

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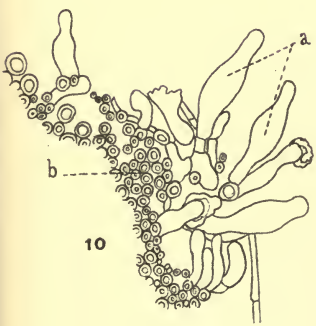

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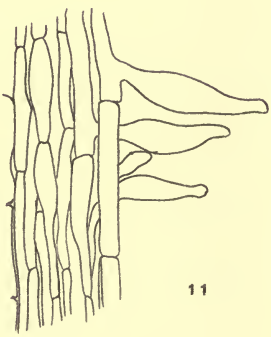

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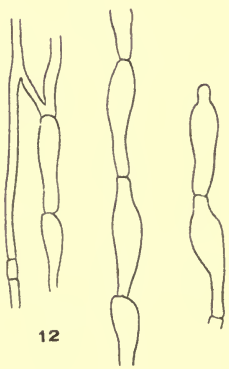

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XXIX. SYMBIOSIS IN THE GENUS LOLIUM.

E. M. Freeman.

The existence of fungus hyphæ in *Lolium temulentum*, *L. perenne* and *L. italicum* furnishes interesting relationships between host and parasite. Nothing has as yet been done with the two latter species but the life-cycle of the *Lolium temulentum* fungus is perhaps completely known. There is however no reason for supposing that they differ at all from *L. temulentum* in their life-histories. It cannot be affirmed without reservation that the entire life-history of *L. temulentum* is understood, but it can be affirmed that the yearly life-cycle is known, and that the parasite can live on indefinitely, infecting generation after generation of *Lolium* plants without spore intervention.

The facts of the life-history are briefly as follows: certain grains of *L. temulentum*, varying in number in ordinary commercial mixtures of the grains, from 85 per cent. to 98 per cent. of the total number, show a layer of hyphæ in the hyaline layer just exterior to the aleurone cells. Occasionally these hyphæ penetrate into the endosperm, between the aleurone and starch cells, but never enter them. The fungus does not apparently thrive in the endosperm. On the convex side of the grain the hyphae extend almost or quite to the tip of the scutellum but never enter the scutellum from this point. Along the grain groove the hyphæ are missing except at the very base where an infection patch exists, from which hyphæ can be found penetrating into the base of the scutellum of the embryo and from here to the growing point of the plumule, where a considerable patch of mycelium is developed and remains dormant until the grain germinates. On germination the hyphæ keep pace in their growth with that of the growing point and can be found here throughout the remaining life of the plant. The hyphæ develop in all of the branches and also in the leaf-bases. The appearance in the latter is explained in the similar chemotactic properties of the basal leaf-meristem to those of the stem growing-point. In the young ovaries the hyphæ permeate the nucellus and here develop luxuriantly. They are pushed back by the

elongation of the embryo-sac and at the time of fecundation of the egg the hyphæ along the funicular region have ceased growth, thus causing the isolation of a patch of hyphæ between the point of the attachment of the ovule and the micropyle. This later becomes an infection-layer, for from this patch arise those hyphæ which penetrate the embryo. The hyphæ do not enter the embryo until the latter shows the rudiments of the scutellum and growing point. As soon as the latter is established hyphæ grow from the infection-layer directly to the growing point and by the time the grain is mature have built up a patch of mycelium. Thus the mycelium passes from generation to generation of the host by direct infection and is apparently able to dispense with spore formation.

Since these facts were established I have attempted in various ways, by altering the conditions of growth of the fungus to induce it to form spores but all efforts have as yet failed. The most obviously probable way of succeeding in this attempt seemed to be the following. If the growth of the young embryo were prevented the fungus might establish an extraordinarily vigorous mycelium and in the absence of the embryo might revert to the spore-forming habit, for it would seem probable that the ancestral form might have formed its spores or a sclerotium in the ovary. In one respect expectations were realized, for a vigorous mycelium in the nucellus was produced. In fact the hyphæ were so densely compacted as to constitute a sclerotium-like body; but this was very small. Whether or not the fungus inhibited the production of the normal amount of nucellar tissue was not to be determined, but could now be ascertained since plants with and plants without the fungus can be raised at will. However the fungus refused to form spores. Two explanations might be offered for this failure: (1) the habit of mycelial infection may be so well established that ability to form spores has been lost entirely; (2) the fungus may be some ergot-forming parasite or one which forms spores in some other organ of the host plant. Analogy with other forms however indicate the greater probability of the first proposition.

As the development of some parasitic fungi seems to be favored by weakness in the host plants, *L. temulentum* plants with the fungus were partially starved by retention in dark chambers for certain periods. It was hoped that the consequent etiolation would favor the parasite at the expense of the host.

This experiment also failed and a consideration of the nature of the fungus might lead one to predict this result. Very closely adapted parasites thrive best on healthy plants, so that during the vegetative period conditions favorable to the host would favor the parasite also. Whatever the true nature of the *Lolium* fungus or its ancestors may be, there is no doubt that it is now a very highly specialized parasite. Consequently the weakening of the host did not favor a tendency toward spore formation, in the predominance of parasite over host. These experiments therefore only strengthen the supposition that the spore forming power of the fungus has disappeared entirely.

Further cultural investigations have confirmed the result of previous anatomical research on the life-history of the fungus. Twelve plants of *L. temulentum* with the fungus were planted in pots of three each at the Cambridge Botanical Gardens in Cambridge, Eng., and six were left in the open while six were placed under glass bell jars during the flowering season. This precaution was taken to guard against the possibility of the transferrence of fungus spores with the pollen. It should be mentioned here however that no fungus hyphæ have been found in any part of the stamens except at the base as in other leaves. Twelve plants without the fungus were placed under similar conditions. The entire crops of these plants were sent to me in the fall of the same year. The number of grains received in each case was as follows:

From plants with the fungus in the open	3,596
From plants without the fungus in the open	222
From plants with the fungus covered at flowering	1,071
From plants without the fungus covered at flowering	824

Out of these 100 of each were examined to determine the presence of the fungus. In every case 100 per cent. came true to the parent plant. Of another lot of with-fungus plants 100 grains were examined and again 100 per cent. were found infected. This should establish beyond a reasonable doubt both the accuracy of this method of infection and the existence of two races of *L. temulentum* one with and the other without a fungus and further supports my previous researches on the modus operandi of infection.

It is a very noticeable fact that the crops of these Cambridge plants show a considerable difference in the number of grains produced by the two races and that the plants with the fungus

seem to be the more vigorous of the two. From the seeds of this crop I planted this last summer an approximately equal number of with- and without-fungus grains under conditions as nearly alike as possible. The number of grains obtained was not determined but the experiment was undertaken merely to observe the growing plants.

Both races seem to thrive well but the with-fungus plants were without doubt on the average more vigorous. All observers who have worked with *L. temulentum* agree that the fungus exercises no noticeably injurious effect upon the host. The above comparisons indicate that not only is this true but that we have here experimental evidence of a case of true symbiosis — a symbiosis differing in many respects from mycorrhizal symbiosis and the symbiosis of the lichens. This condition is not so remarkable when we consider the well-known cases of those grass smuts where the presence of the fungus is not betrayed until spore formation. In fact from the analogy of stimulation of many rusts as well as smuts in hypertrofication, etc., stimulation of the *Lolium* plant would almost be expected. The later destructive action in spore formation is here unnecessary on account of the new device for infection. One may therefore consider that this symbiosis has arisen through a previous condition of destructive parasitism.

The nature of the fungus still remains an open question. I have previously enumerated the objections to the assignment of this fungus to the ergot-forming parasites and it certainly has little or no resemblances to the Uredineæ. Nor has it any similarity to the Hyphomycetes and Pyrenomycetes of molded grains. The Ustilagineæ seem to furnish the closest affinities. The growth of the hyphæ in the growing point and the infection of the nucellus are quite similar to certain smuts. The hyphæ seem to be intercellular and in no case was I able to demonstrate the penetration of the cell walls either by the ordinary hyphæ or haustoria. In this respect and in the abundance of septations in the nucellar hyphæ the fungus seems to differ from smuts. In regard to the latter point the fungus seems at this stage to have departed from the normal ustilagine methods and the septations may perhaps be regarded as relics of those septations preceding the chlamydospore formation. It is well known that oat smut infects seedlings. And it would not be unnatural to expect a still earlier infection. But if this were

pushed further forward it must necessarily take place in the intraseminal life of the host. Granting this to be the case, one would expect to find the infection finally taking place as soon as the stem growing point is established, for not until then are the chemotactic substances present to attract the hyphæ. And this is what actually occurs.

An analogous driving forward of the period of infection has probably taken place among those parasites attacking the host plant in the extraseminal seedling stages. It seems not improbable that the oat smut may have formerly been able to infect any immature tissues of the host as the corn smut does. The ovary is a particularly favorable organ both on account of nutrition and distribution facilities and hence, the preference for the ovary as a spore-bearing region for the parasite. The establishment of an attraction by the growing point of the stem and the localization of the sporiferous region in the ovary would naturally be followed by an earlier infection since the presence of the fungus in the unbranched stem multiplies the results of the infection when the stools are mature. The proximity of spores to the grains in sowing becomes obviously advantageous and hence the infection would be pushed forward to the earliest stages of the extraseminal development of the host, as in the oat smut. If now, one presupposes a chemotactic attraction of the growing point for the hyphæ of a smut and also at the same time a failure of the fungus to destroy the endosperm and young embryo during spore formation, then nucellar hyphæ crowded back by the endosperm and embryo, might easily effect direct infection of the growing point of the young embryo. This period would then be shoved forward to the formation of the earliest rudiments of the growing point. It seems probable that the fungus is therefore an ustilagine which has brought forward its infection period to the intraseminal stages of the host and more particularly to the first appearance of the growing point, and has lost the power of spore formation.

Apparently infection of a without-fungus plant of *L. temulentum* is impossible as is also the eliminating of the fungus from the with-fungus plants, barring the possibility of failure to infect all ovaries in an inflorescence, which case seems highly improbable. There exists therefore not only two races of *L. temulentum*, but also of *L. perenne* and of *L. linicola* and probably of other species of *Lolium*, in each case one with, and the other without, the fungus symbiont.

The question now naturally arises whether the symbiosis was established previous to or subsequent upon the origin of these species. In other words has the symbiosis arisen once or three times ? The simpler explanation seems at first to be that the symbiosis was previous. This would however presuppose the development in each of the three species of two parallel lines one with and one without the fungus. On the other hand a subsequent establishment of the symbiosis would require like results for the same fungus or for several closely related fungi attacking three species of *Lolium*. A fuller knowledge of the inter-relations between these species as well as of the remaining species of *Lolium* is necessary before an intelligent opinion on this point can be framed.